Dear Dr. Ardón and Anonymous Reviewer #4,

We very much appreciate the time and effort you put into reviewing this manuscript as well as the other two reviews and our extensive responses to them. We will respond to your comments in a point-by-point fashion below, and we have attached the revised manuscript as a supplement with the changes suggested by Dr. Ardón marked in red text and those suggested by Reviewer 4 highlighted in yellow. Again, we appreciate the feedback and recognize that your efforts have resulted in an improved manuscript.

Respectfully,

Carmella Vizza, Will West, Stuart Jones, Julia Hart, and Gary Lamberti

RESPONSE TO REVIEWER 3, DR. MARCELO ARDÓN:

I think this is an interesting study. The authors did a good job responding to the two previous reviewers. I have some minor comments regarding parts that could be clarified or worded differently.

Comment 1: Page 1 Line 20 of the abstract- I think the comma should be moved to after the word added. **Response:** We appreciate you catching this error. We have corrected it on **pg. 1, line 20.**

Comment 2: Page 2 line 10-12- this sentence is too long. Break it up into 2 sentences, and check your comma placements. I don't think you need a comma after the word fertilization. **Response:** We agree that we could make this sentence easier to read by breaking it up into two, more clearly constructed sentences. Please see **pg. 2, lines 6-9.**

Comment 3: Page 2- line 25- I would change the word "signal" to something like "lead to" **Response:** Thank you for this suggestion. We have made this change on **pg. 2, line 19.**

Comment 4: Page 3- lines 2-3- I would remove the phrase "which may be influenced by rising global CO2 concentrations and temperatures". You already spent two paragraph explaining these mechanisms. **Response:** We have removed this phrase on **pg. 2, lines 26-27.**

Comment 5: Page 4- 16- the brackish tidal wetlands seem to vary more than the freshwater wetlands. It seems like your brackish wetlands span a large salinity gradient, even overlapping the freshwater wetlands. Why combine them? **Response:** Originally, we chose not to combine freshwater systems in presenting Table 1 because we had more extensively characterized them, and as wetland ponds, they have more clearly delineated boundaries. In contrast, brackish tidal wetland sites were located within one large continuous area. However, based on your comments and those of reviewer 4, we have decided to add in all 10 brackish sites in both **Table 1 and 2**. Nevertheless, we should make it clear that brackish tidal wetlands sites along the salinity gradient were not combined for analyses. Please see **pg. 4, lines 11-12 and 19-20**, where we have attempted to make this clearer.

Comment 6: Page 4 line 28 – "We then we"- please correct **Response:** We appreciate you catching this typo. We have

corrected it on **pg. 4, line 28**.

Comment 7: Page 4 lines 28-30- I am confused by the number of samples in your comparisons. You sampled 9 separate locations in the non-tidal freshwater water wetlands and 5 locations in your brackish wetlands. But then you did 5 incubations per location. So is that n=45 for the non-tidal freshwater wetlands, and n=25 for the brackish wetlands? Please clarify. **Response:** We have clarified this in **pg. 4, lines 24-30**.

Comment 8: Page 5- line 15- can you give us a quick summary of the differences in the quality of these species? Is the Tiegs data from the same time you did your experiment? **Response:** We have added a summary of the quality differences in **pg. 5, lines 19-21**. The Tiegs data was collected a few years earlier than our experiment, but in the same study area.

Comment 9: Page 8 line 29- where the %C, %N, %P, C:N, and C:P measured in this experiment, or are they from the Tiegs et al. 2013 paper? **Response:** These data were from the Tiegs et al. 2013 paper. We have clarified this on **pg. 8, line 30**.

Comment 10: Page 9 lines 4-5- It is not clear to me how I can assess that ecosystem differences are bigger than temporal differences from Tables 1 and 2. You don't give any measure of temporal variability for any of the wetlands in the tables. The variation in brackish wetlands could be spatial or temporal. And as I pointed out above, it seems strange to me to combine the brackish wetlands when the ranges are bigger than the non-tidal freshwater wetlands, which you say you kept separate due to spatial differences. **Response:** Brackish wetlands were not combined in statistical analyses, and we have added all 10 brackish sites in both **Tables 1 and 2**. Tables 1 and 2 contain both spatial and temporal variation, but you are correct that one cannot deduce from those tables alone whether ecosystem differences are bigger than temporal differences. Instead we specifically break out ecosystem and temporal variation in the statistics that follow this opening sentence. We will attempt to rephrase it to clear up any confusion. Please see **pg. 9, lines 6-7**.

Comment 11: Page 11 lines 5 and 6- what do you mean by "the interaction of global change mechanisms"? Acetate and sulfate availability are not "global change mechanisms". Please explain. **Response:** We have attempted to clarify this by specifically listing global change mechanisms that could affect acetate and sulfate, please see **pg. 11, lines 9-14**.

Comment 12: Page 11 line 16- again I get distracted by how you use the word "signals". Leads to or indicates? I am not quite sure what you mean in this sentence. **Response:** Thank you for this word choice suggestion, please see **pg. 11, line 24** where we use the indicate instead of signal.

Comment 13: Page 11 line 30- add a comma after organisms. **Response:** Thank you for this suggestion, we have added a comma on **pg. 12, line 8**.

Comment 14: Page 12 lines 1-2- calcium co-precipitating with P does not necessarily lead to ammonium release. But increased salinity does lead to ammonium release through cation exchange. **Response:** Yes, we agree with you and have changed the sentence to better reflect that calcium co-precipitation with P does not necessarily have a causal relationship with ammonium release. Please see **pg. 12, line 10.**

Comment 15: Page 13 lines 14-16- Can you provide a citation for these patterns? I don't think you can infer all these patterns from your 2 time sampling. **Response:** We can only provide a general citation for how plants and their phenology can affect ecosystem process. In addition to adding this general citation, we have changed the wording to make it clear that the seasonal trajectory we are proposing, while it fits with our data, is hypothetical. We added in this hypothesis in the first revision submitted to BGS due to the fact that another reviewer felt our explanation for the seasonal patterns was dissatisfying. Please see **pg. 13, lines 22-25**, where we have attempted to incorporate your comments, while still offering a more satisfying explanation for the other reviewer. We will defer to the editor here as to whether we have struck the right balance between the two sets of reviewer comments about this discussion paragraph.

Comment 16: Page 13 line 30 130-320 is not two orders of magnitude larger than 5. It is one order of magnitude. **Response:** Thank you for this suggestion. The data are between 1-2 orders of magnitude larger than 5, but not 2 exactly. We have changed the wording on **pg. 14, line 9**.

Comment 17: Page 16 line 25- why CO2 fertilization? I would remove that one. Or change it to say increased organic matter to be consistent with your Fig 1. Increased organic matter can be caused from longer growing season, CO2 fertilization, and increased breakdown of soil organic matter. **Response:** Thank you, we have changed it be more consistent with Fig. 1. Please see **pg. 17, line 5**.

RESPONSE TO REVIEWER 4:

As the third reviewer of this manuscript I had an opportunity to read the previous reviews and responses. The authors addressed each of the previous comments in detail and made changes to the manuscript, with the result that the manuscript that I received has clearly improved. My comments are of two varieties, reactions to the authors responses to previous comments and new comments from my own review. I am only commenting on the responses that I had some objection to, so let me state at the beginning that the authors did a good job of addressing the many other comments that I do not mention.

Comment on Previous Responses 1: Dr. Scranton objected to the authors description of their seawater addition experiments as a sea level rise manipulation. I agree with Dr. Scranton that this term in inappropriate because it conjures up a large number of interacting processes and feedbacks that were not manipulated or observed in this study. Changing the term to "biogeochemical sea level rise" brings it closer to the reality, but even this term is misleading. I suggest "seawater addition experiment" or "short-term seawater addition experiment" to efficiently communicate what the authors did. The connection between the seawater addition experiment presented here and the far more complicated issue of sea level rise will be immediately obvious to most readers, particularly in the context of the introduction to the paper. This change should be made throughout the full paper. **Response:** Thank you for this suggestion. We have made this change throughout the entire manuscript whenever we refer to what we formerly called the "biogeochemical effects of sea-level rise simulation." We still

refer to sea-level rise as a global change mechanism in both the introduction and discussion, but only as it applies to broader scale patterns.

Comment on Previous Responses 2: I object to the term "greenhouse gas compensation", but for somewhat different reasons than Dr. Scranton. My reason is that the current greenhouse gas balance of any ecosystem is irrelevant to the issue of climate change because that balance (regardless of closeness to the compensation point) is part of the greenhouse gas "baseline". It does not matter if the system is close to or far from the compensation point provided that balance is about the same now as it was in the past when climate was more stable. In the context of this paper the authors are trying to make a different point, which is that factors such as temperature and elevated CO2 cause this balance to change from the baseline. Please drop the "compensation" idea and emphasize the idea of change from the baseline. The most direct way of communicating this idea is to use the term "radiative forcing". **Response:** We understand your objection and appreciate the clarification. We have removed mention of compensation point and instead refer to change from the "wetland greenhouse emissions baseline." Please see **pg. 2, lines 4-9**.

Comment on Previous Responses 3: Dr. Scranton commented on the amount of text devoted to elevated carbon dioxide and warming. I am happy that these issues are discussed in the paper, but I agree that it creates an expectation in the introduction that CO2 and temperature were manipulated in the study. This problem can be avoided by generalizing the context of the present study from elevated CO2 and temperature to include any factor that changes carbon uptake, carbon loss as CO2, or carbon loss as CH4. Elevated CO2 can then be introduced as one example of a factor that can change plant growth rates, and temperature as another example. For example, something like this: "Any factor that changes the availability of electron donors (i.e. organic carbon) or electron acceptors (e.g. sulfate supply) has the potential to change the greenhouse gas balance of an ecosystem, thereby exacerbating or mitigating radiative forcing of climate. Factors that are known to change organic carbon availability include elevated CO2 and temperature, which affect both plant physiology and potentially growing season length. Factors that can alter the supply of specific electron acceptors include sea level rise (i.e. sulfate supply) and agricultural pollution (i.e. nitrate supply). We investigated the sensitivity of CH4 production to changes in the supplies of organic carbon (electron donor) and sulfate (electron acceptor) in wetland soils. Our objective was to gain mechanistic insights on a subset of factors that regulate CH4 emissions in wetland systems that will transition from non-tidal and tidal freshwater to brackish with climate change and sea level rise." Again, please discuss your manipulations as "saltwater additions" and limit the mention of sea level rise except to give some occasional context. **Response:** We appreciate your suggestions and have revised our second paragraph in light of this comment. Please see **pg. 2, lines 11-25**.

Comment on Previous Responses 4: Dr. Scranton has a comment in reference to P2, Line 16 of the original submission where he points out that a difference in sulfate concentration does not translate into a difference in redox conditions. I agree and would add that "redox conditions" is mostly used incorrectly in the paper. I suggest that the term "redox" be dropped

almost entirely because there are no redox data in the paper and it is not possible to infer redox state from these data. Instead use the term "electron acceptor availability". **Response:** You are correct in that we did not directly measure redox potential. However, it is not uncommon in the literature to infer some information about redox conditions from the presence of certain chemical species. Nevertheless, we believe that "electron acceptor availability" is a more precise term, and we have made this change throughout the rest of the manuscript.

Comment on Previous Responses 5: Please refine your edits in response to Dr. Neubauers comment about the word "generally" in describing the CH4 production response to litter addition. I think his point was that the total absence of such a response in two species is the more important result. Please edit to read something like "Organic matter addition consistently stimulated CH4 production rates for just two of the four species used as amendments. This indicates that the consequences of changes in plant production on CH4 production will be highly species dependent." **Response:** Thank you for clarifying this. We have made a change to better reflect this on **pg. 11, lines 7-9**.

Comment on Previous Responses 6: I understand the authors point that non-tidal sites are easily delineated from one another but the tidal sites are not, but I cannot understand why this must translate into the structure of tables 1 and 2. Because the brackish sites are used as independent replicates the reader needs to know how they differed from one another with respect to the characteristics in tables 1 and 2. Please list each brackish site separately. **Response:** There were a total of ten incubations (5 sites along a salinity gradient x 2 time periods) conducted at the brackish tidal wetlands, and sediment/water was sampled from one continuous wetlands complex. Because brackish tidal wetlands were characterized less than the freshwater wetlands, we originally grouped them together in a summary form in Tables 1 and 2. However, we did analyze each brackish site separately, and in light of that and your suggestion, we have added the data for each site in **Tables 1 and 2.**

Comment on Previous Responses 7: Specific Response 22. Although the response answers the question the way it was phrased, I believe the point of the comment was that the authors make too big a leap from the result that acetate and sulfate correlations behaved differently in the two wetland types to "multiple global change mechanisms". Please make a more modest statement such as "This indicates that we do not have a sufficient mechanistic understanding of how changes in electron donor and acceptors will interact to ultimately influence methane production." **Response:** Thank you again for the clarification. Please **pg. 11, lines 9-14,** where we have made this change.

New Comment 1: P3, L1. I respectfully disagree that we need to understand the effects of sea level rise on CH4 production to forecast the global CH4 budget. Sea level rise is an issue that affects wetlands that are already tidal or will be tidal in the future, and wetland carbon budgets like those of Bridgham et al. (2006 in Wetlands) show that CH4 emissions from such wetlands are a small part of the methane coming from wetlands globally. Perhaps something like "To accurately forecast the effects of sea level rise on coastal wetland greenhouse gas budgets requires a process-level understanding of responses to

potential changes in electron donor and acceptor availability." **Response**: Thank you for this suggestion. Please see **pg. 2, lines 26-27**, where we have made this change.

New Comment 2: P16, L8. The most likely explanation for the negative correlation between CH4 production and porewater acetate is that the added porewater lowered pH. This is a very common artifact in incubation studies that can only be dismissed with data on the pH of the incubation water. It can happen in two ways: (1) is if the added porewater is lower in pH than the incubation water or (2) if the porewater stimulates microbial respiration, causing CO2 to accumulate in the jar and acidify the incubation water. The correlation between CH4 and acetate could indicate that porewater with more acetate also had lower pH; this is possible because acetate is a weak acid. Alternatively, porewater with more acetate could have acidified the sample faster due to more CO2 production. This comment has the potential to affect other parts of the discussion as well. **Response:** Although we acknowledge that pH can affect CH_4 production, we respectfully disagree with the reviewer on this point. We did not add any additional porewater to the incubation; porewater was extracted from a subsample of the sediment prior to incubation. Therefore, porewater measurements represented what was naturally in the sediment. Nevertheless, we did add water from the overlying water column in the incubation (please see **Table 1** for chemistry information), and while it is possible that this altered the pH of the incubation, we were not able to detect any acetate in the water column. Therefore, we find no reason to suspect that the incubation pH was correlated with the acetate concentration of the porewater, which is also the total concentration in the incubation before adding organic matter. If we had also found a negative correlation between CH_4 production and porewater acetate in the brackish/freshwater wetland comparison, then the pH explanation might be an alternative explanation. However, we found that porewater acetate had a positive relationship with CH_4 production in incubations where no organic matter was added. It was not until we added organic matter that we actually observed this negative relationship between the change in CH_4 production and acetate, which is why we think the amount of electron donors for methanogens became saturated. Additionally, we believe that sulfate is more likely to affect the pH of the incubation solution than acetate because the total sulfate concentrations in the incubation were much higher than the acetate concentrations and because a hydrogen sulfate ion is a stronger acid than protonated acetate.

New Comment 3: P2, L20. Delete "of choice" **Response:** Done, please see **pg. 2, line 14**.

New Comment 4: P2, L24. It is incorrect to say that "carbon" (or carbon dioxide, which is what I think the authors meant) is not an energetically favorable electron acceptor because this can only be determined by calculating the energy yield of full redox couples. You can say that "CO2 reduction is not an energetically favorable electron accepting pathway compared to other pathways." **Response:** We agree with you that generally one should not consider the electron acceptor without the donor. Please see **pg. 2, line 17**, where we have attempted to correct this. Also, carbon in the form of a methyl group (CH_3)

or carbon dioxide acts as an electron acceptor in acetoclastic or hydrogenotrophic methanogenesis, respectively. Please see **pg. 2, line 18**, where we have attempted to clarify this.

New Comment 5: P2, L25. Replace "signal" with "indicate" **Response:** Done, please see **pg. 2, line 19**.

New Comment 5: P2, L30. Edit to "Both electron donor and electron acceptor availability will therefore play an important role in determining the effects of global change on CH4 production." **Response:** Done, please see **pg. 2, lines 24-25**.

New Comments 6 & 7: P3, L18. Edit to "periodically inundated tidal brackish wetlands". P4, L4. Tidal wetlands are not typically inundated at the lowest tides, almost by definition. Does this refer to mean tide? Mean low tide? **Response:** These tidal wetlands are at a confluence of a river mouth and the Gulf of Alaska, so do they do tend to be fully inundated or water-logged even at mean low tide. We have attempted to make this clearer, please see **pg. 3, line 29, and pg. 4, lines 1-3**.

New Comment 8: P8, L19. Edit to "…were affected by being incubated anaerobically with tidal…" **Response:** Done, please see **pg. 8, line 20.**

New Comment 9: P9, L17. A number of reviewer comments boil down to distinguishing between cause and effect in the language of the paper. This is one example where the word "influenced" should be replaced by "correlated with" or "associated with". You cannot say that acetate influenced CH4 production. **Response:** Done, please see **pg. 9, lines 18-19**.

New Comment 10: P10, L5. Edit to "Incubating non-tidal freshwater wetland soils with brackish water…" **Response:** Done, please see **pg. 10, line 9**.

New Comment 11: P12, L22. Change "coupled" to "related" because coupled implies you separated cause and effect. **Response:** Done, please see **pg. 12, line 30**.

New Comment 12: P14, L10. Please use a more precise word or phrase in place of "primed" **Response:** Done, please see **pg. 14, lines 19-20**.

Respectfully,

Carmella Vizza, Will West, Stuart Jones, Julia Hart, and Gary Lamberti

Regulators of coastal wetland methane production and responses to simulated global change

Carmella Vizza[1], William E. West[1, 2], Stuart E. Jones[1], Julia A. Hart[1, 3], and Gary A. Lamberti[1]

[1]Department of Biological Sciences, University of Notre Dame, Notre Dame, 46556, USA
[2]Kellogg Biological Station, Michigan State University, Hickory Corners, 49060, USA
[3]Center for Limnology, University of Wisconsin, Madison, 53706, USA

Correspondence to: Carmella Vizza (cvizza@nd.edu)

Abstract. Wetlands are the largest natural source of methane (CH_4) to the atmosphere, but their emissions vary along salinity and productivity gradients. Global change has the potential to reshape these gradients and therefore alter future contributions of wetlands to the global CH_4 budget. Our study examined CH_4 production along a natural salinity gradient in fully inundated coastal Alaska wetlands. In the laboratory, we incubated natural sediments to compare CH_4 production rates between non-tidal freshwater and tidal brackish wetlands, and quantified the abundances of methanogens and sulfate-reducing bacteria in these ecosystems. We also simulated seawater intrusion and enhanced organic matter availability, which we predicted would have contrasting effects on coastal wetland CH_4 production. Tidal brackish wetlands produced less CH_4 than non-tidal freshwater wetlands probably due to high sulfate availability and generally higher abundances of sulfate-reducing bacteria, whereas non-tidal freshwater wetlands had significantly greater methanogen abundances. Seawater addition experiments with freshwater sediments, however, did not reduce CH_4 production, perhaps because the 14-day incubation period was too short to elicit a shift in microbial communities. In contrast, increased organic matter enhanced CH_4 production in 75% of the incubations, but this response depended on the macrophyte species added, with half of the species treatments having no significant effect. Our study suggests that CH_4 production in coastal wetlands, and therefore their overall contribution to the global CH_4 cycle, will be sensitive to increased organic matter availability and potentially seawater intrusion. To better predict future wetland contributions to the global CH_4 budget, future studies and modeling efforts should investigate how multiple global change mechanisms will interact to impact CH_4 dynamics.

Keywords: Methanogenesis, sea-level rise, saltwater incursion, redox, microbial communities

1 Introduction

Wetlands contribute about 60% of all natural methane (CH_4) emissions to the atmosphere (Kirschke et al., 2013). As global temperatures continue to increase, some models predict that wetland CH_4 emissions will double by 2100 (Gedney et al., 2004). Because CH_4 is a potent greenhouse gas whose radiative forcing continues even after its oxidation to CO_2 (Neubauer and Megonigal 2015), higher wetland emissions could trigger a positive feedback loop that further increases temperatures and CH_4 release. Higher future CO_2 levels could result in further warming, an extended growing season (Walther et al., 2002), and CO_2 fertilization of photosynthetic plants (Matthews, 2007; Ringeval et al., 2011). If the resulting increases in plant productivity provide additional organic matter to fuel additional CH_4 production, this effect could shift the wetland greenhouse gas emission baseline. Predicting the response of these ecosystems to global change is challenging because we do not fully understand the sensitivity of the CH_4 cycle to enhanced productivity of wetland plants (McGuire et al., 2009; Ringeval et al., 2011).

Any global change element that directly alters the availability of electron donors or electron acceptors could change CH_4 production rates and baseline emissions, thereby exacerbating or mitigating the radiative forcing of climate. Methanogens generally use substrates provided by the fermentation of organic matter as electron donors, producing CH_4 via two pathways: (1) acetoclastic methanogenesis, where acetate is electron donor and acceptor, and (2) hydrogenotrophic methanogenesis, where H_2 and CO_2 are the substrates utilized (Conrad, 1999). Acetate is therefore an important substrate that methanogens either directly use (acetoclastic pathway) or indirectly use via the H_2 and CO_2 resulting from its fermentation and that of other organic matter (hydrogenotrophic pathway). However, assuming competition for the same electron donor, methanogens can be outcompeted for these substrates because carbon as CH_3 or CO_2 is not an energetically favorable electron acceptor in comparison to those used by other microbes (e.g., NO_3^-, SO_4^{2-}). The presence of alternative electron acceptors can indicate intense microbial competition for the fermentative substrates that methanogens utilize (Lovley and Klug, 1983; 1986; Lovley and Phillips, 1987). For example, Winfrey and Ward (1983) observed much greater rates of sulfate reduction than CH_4 production in intertidal sediments until sulfate became depleted. However, an abundant supply of organic matter can increase substrate availability, act as an electron donor, and allow for depletion of alternative electron acceptors (Achtnich et al., 1995). Both the availability of electron donors and acceptors will therefore play an important role in determining the effects of global change on CH_4 production.

Accurately forecasting the effects of sea-level rise and increased organic matter on coastal wetland greenhouse gas budgets requires a process-level understanding of responses to potential changes in electron acceptors and donors (Fig. 1). Laboratory studies and field surveys report increased CH_4 production and emissions with warming (Moore and Dalva, 1993; Klinger et al., 1994; Lofton et al., 2014). Additionally, elevated CO_2 levels can also lead to higher photosynthesis and CH_4 emission rates (Megonigal and Schlesinger, 1997; Vann and Megonigal, 2003). However, despite their potential importance in regulating CH_4 emissions from wetlands, especially those at northern latitudes, few studies have attempted to simulate the

effects of seawater intrusion or increased substrate availability on CH_4 production. Both of these global change mechanisms are likely to disrupt coastal wetland biogeochemical cycles, especially at northern latitudes where their effects are likely to be stronger and more abrupt.

We studied wetland ecosystems in the Copper River Delta of Alaska, an area vulnerable to global change because of its northern location and proximity to the ocean. Over the past 50 years, average annual temperatures in Alaska have increased 1.9 °C, with winter temperatures rising 3.6 °C (U.S. Global Climate Change Program, 2009), which is extending the growing season. In addition, the projected global sea-level rise of 100 cm by 2100 (Vermeer and Rahmstorf, 2009) will be exacerbated along the southcentral Alaskan coast where tectonic subsidence is prominent (Freymueller et al., 2008). For example, the Copper River Delta, which is subsiding at about 0.85 cm per year (Freymueller et al., 2008), is at risk of a relative sea-level rise of about 170 cm by 2100.

Our study objectives were to (1) compare CH_4 production rates and microbial community abundances in sediments from constantly inundated non-tidal freshwater and tidal brackish wetlands on the Copper River Delta, (2) simulate seawater intrusion in freshwater wetlands using a seawater addition experiment, and (3) simulate increased organic matter availability in freshwater wetlands. We hypothesized that (1) tidal brackish wetlands sediments will have lower CH_4 production rates than those from the non-tidal freshwater wetlands, (2) tidal brackish wetland sediments will have higher abundance of sulfate-reducing bacteria, but lower numbers of methanogens than non-tidal freshwater wetlands, (3) simulating seawater intrusion in freshwater sediments will decrease CH_4 production rates, with sulfate availability largely being responsible for this effect, and (4) increasing the amount of organic matter available will enhance CH_4 production, but substrate quality will moderate this effect. Our conceptual model for these interactions is depicted in Fig. 1.

2 Materials and Methods

2.1 Study area

The Copper River in southcentral Alaska is the eighth largest river in the United States (U.S. Geological Survey, 1990). Draining a large region of the Chugach Mountains and the Wrangell Mountains into the Gulf of Alaska, the Copper River and its sediment deposits have shaped the largest contiguous wetland on the Pacific Coast of North America. The Copper River Delta (CRD) encompasses about 283,000 hectares of wetland habitat and supports extraordinary biodiversity (Bryant, 1991) in a relatively pristine landscape. Wetlands and shallow ponds (0.2 to 2 m in depth) were created and modified by the Great Alaska earthquake in 1964 that elevated the CRD by 1−4 m depending on location (Thilenius, 1995). A natural succession of wetlands thereby emerges from the ocean to the uplands (Fig. 2). Our study focused on the brackish tidal wetlands and non-tidal freshwater wetland/pond habitats. The brackish tidal wetlands we chose to study are at the confluence of a river mouth

and the Gulf of Alaska. Therefore, these wetlands become increasingly brackish and deeper during rising high tide, but are also waterlogged during mean low tide; we consider them appropriately comparable to the fully inundated non-tidal freshwater wetlands. The freshwater wetland habitats currently receive little to no tidal influence, but their surrounding sloughs and rivers are tidally influenced, which could result in future seawater intrusion with sea-level rise. We consider the freshwater wetlands to be "pond-like" because they have clearly delineated boundaries, whereas the brackish wetlands are more continuous in nature. We chose these two ecosystem types because they are the most prevalent yet distinctive habitats on the CRD with which to contrast CH_4 production.

2.2 Experimental design

2.2.1 Sample collection

Using a handheld bucket auger, sediment samples (~ 250 mL) were collected from nine non-tidal freshwater wetlands and five tidal brackish wetland sites varying in physicochemical parameters (Tables 1 and 2). Because non-tidal freshwater wetland ponds had distinct boundaries and extensive habitat heterogeneity within each wetland (i.e., open water and several different macrophyte zones), we collected at least five sediment samples representative of the different habitats at each wetland (n = 9) along with at least 1 L of hypolimnetic water during each sampling period, so that the average CH_4 production rates from each system could be accurately assessed. In contrast, the tidal brackish wetland complex was continuous, lacking distinct boundaries, and generally exhibited less habitat heterogeneity than the non-tidal freshwater wetlands (i.e., we observed only sites dominated by *Carex* spp.). Because we observed temporal fluctuations in salinity with a YSI Pro Plus multiparameter water quality meter indicative of tidal influence, we collected 1 L of water and one sediment sample at five different sites along a salinity gradient. Although sediment and water from tidal brackish wetland sites were collected in one continuous wetland complex, they were considered separately in analyses due to large differences in salinity.

2.2.2 Non-tidal freshwater and tidal brackish wetland comparison

To assess CH_4 production, laboratory incubations were conducted using sediment and water samples collected during two sampling periods (June and August 2014). To capture the greater habitat heterogeneity of the non-tidal freshwater wetlands, we conducted five CH_4 production assays for each wetland (5 sediment samples x 9 wetlands x 2 time periods = 90 total incubations). We therefore characterized the non-tidal freshwater wetlands to a greater spatial extent than the brackish tidal wetlands where we conducted ten total incubations (5 sites along a salinity gradient within the continuous tidal brackish wetlands complex x 2 time periods). To account for this difference in spatial sampling, we then used the average CH_4 production rates from each non-tidal freshwater wetland as a replicate in comparing CH_4 production rates between non-tidal freshwater (n = 9) and tidal brackish (n = 5) systems at each sampling period.

2.2.3 Seawater addition experiment

To assess the effects of seawater addition on CH_4 production, additional sediments were collected in June from a single site in five of the freshwater wetlands (n = 5) and then incubated with tidal brackish water (6.3 mM sulfate). We then compared them to the average CH_4 production rates of the five sediment samples incubated with freshwater from that same subset of non-tidal freshwater wetlands (n = 5) during June 2014.

2.2.4 Increased organic matter simulation

To assess the effects of increased organic matter on CH_4 production, four sediment samples from different sites were used from five of the non-tidal freshwater wetlands (n = 20). An aliquot of each sediment sample from each wetland was incubated with fresh macrophyte tissue from one of four species (treatment) and then compared to an aliquot that served as a paired control sediment sample (total pairs = 20; 5 wetlands x 4 treatments). This paired design controlled for "within wetland" sediment heterogeneity to better capture the response of the methanogens to adding organic matter, or ΔCH_4 production (treatment–control). Our four organic matter treatments were based upon the four dominant aquatic macrophyte species on the CRD – buckbean (*Menyanthes trifoliata*), horsetail (*Equisetum variegatum*), lily (*Nuphar polysepalum*), and marestail (*Hippuris vulgaris*). Specifically, we cut aboveground tissue to a standard size per species such that 3.0 g of live biomass could be added to each incubation resulting in approximately 0.23 ± 0.02 mmol C per gram of dry sediment (mean ± sd). In most incubations, this addition of organic matter increased the total amount of carbon already available in the sediment by 45 ± 15% (Table 2). All vegetation for each species was collected from the same plant individual to ensure minimal difference in quality within each treatment. Differences in substrate quality between these treatments, as described by % C, % N, and % P as well as C:N and C:P, are available from Tiegs et al. (2013): (1) Horsetail had the lowest carbon content at 38%, while the other three species contain approximately 44–47% C, (2) Lily tissue had the highest % N (2.5) and % P (0.24) followed by marestail with 1.7% N and 0.17% P, and (3) Buckbean had 0.94% N and 0.15% P and horsetail had 1.1% N and 0.11% P.

2.3 Laboratory analyses

2.3.1 Sediment slurry incubations

For each incubation, approximately 60 mL (82 ± 2.5 g) of wet sediment and 60 mL of water were incubated in a 250-mL serum bottle in the dark at approximately 14.0 °C. To remove oxygen introduced to the inundated sediments during sample collection and slurry making, each bottle was made anoxic by purging it with N_2 gas for five minutes. Since incubation temperature was generally lower than average wetland temperature (June: 17.2 ± 0.9 °C, August: 18.4 ± 1.3 °C), estimated rates of CH_4 production potential were considered conservative. However, we do acknowledge that CH_4 production potentials generated by bottle incubations may not exactly reproduce CH_4 production rates in these ecosystems. Headspace samples (10 mL) were removed at 2, 5, 8, 11, and 14 days, injected into a 2-mL serum vial (pre-evacuated with a vacuum pump), sealed with silicone, and stored upside down in water for less than three months until the samples could be analyzed using gas chromatography. To

maintain atmospheric pressure in the slurry incubations, 10 mL of N_2 gas was added after each sampling point. CH_4 concentrations were measured using an Agilent 6890 gas chromatograph equipped with a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA) as detailed by West et al. (2015). After accounting for headspace dilution due to sampling, CH_4 production rates were inferred from the slope of the linear regressions of CH_4 concentrations over time and are reported as nmol CH_4 per g of dry sediment per day (nmol g^{-1} day^{-1}).

2.3.2 Physicochemical measurements

Temperature, pH, dissolved oxygen, specific conductivity, and salinity were measured at each sampling location using a YSI Pro Plus multiparameter water quality meter (YSI, Yellow Springs, OH, USA). Dissolved organic carbon was analyzed using a Shimadzu TOC-VCSH (Shimadzu Scientific Instruments, Kyoto, Japan). All samples, with the exception of five of the tidal brackish samples, registered above the lowest standard (1 mg/L); the five exceptions registered between the blanks and the lowest standard. Acetate, nitrate, and sulfate concentrations were analyzed using a Dionex ICS-5000 (Thermo Fisher Scientific, Sunnyvale, CA, USA), but only sulfate was detectable in the water column. Detection limits for acetate, nitrate, and sulfate were approximately 10, 2, and 1 µM, respectively. Water chemistry analyses were performed using instrumentation at the University of Notre Dame Center for Environmental Science and Technology.

2.3.3 Sediment organic matter and porewater chemistry

To examine starting conditions for each CH_4 production assay, a subsample of sediment was frozen at the start of the incubation for later analysis. A portion of each subsample was dried for at least 48 hours at 60 °C, and the dry weight was recorded. Subsequently, the organic matter in the sediment was combusted at 500 °C for four hours, and the sediment was re-wetted and then dried at 60 °C for at least 48 hours before re-weighing (Steinman et al., 2011). Sediment organic matter was estimated as the percent of sediment material lost during combustion (SOM %) and converted to the total sediment organic carbon (Thomas et al., 2005) available per g of dry sediment (Table 2). To extract porewater from the sediment, another portion (~ 50 mL) was centrifuged for 45 minutes at 4 °C at ~ 4000 RCF. The total volume of supernatant per volume of sediment was recorded, and a subsample of the porewater was also analyzed on the Dionex ICS-5000 for acetate, nitrate, and sulfate. To account for the widely differing porewater volumes we extracted from sediment (0.17 ± 0.09 ml porewater per mL of sediment), porewater concentrations were converted to the total amount of each anion (nmol) per g of dry sediment (i.e., µM x porewater volume in incubation x porewater volume per mL of sediment x sediment volume in bottle / mass of dry sediment x 1000; Table 2).

2.3.4 Microbial analyses

According to the manufacturer's protocol with a PowerSoil DNA isolation kit (Mo Bio, Carlsbad, CA, USA), DNA was extracted from frozen sediments used in other analyses, including multiple June tidal brackish sediments (n = 10), the freshwater sediments used in the seawater intrusion simulation (n = 5), and a composite of the five sediment samples (1 g sediment per sample was added to make a 5-g composite) from the nine freshwater wetlands for the June time period (n = 9).

We chose to make composites for microbial analyses of the non-tidal freshwater wetlands for the purpose of controlling analytical costs while controlling for the significant spatial heterogeneity in these ecosystems. Extracted DNA served as a template for quantitative PCR (qPCR) targeting of two genes – the alpha subunit of methyl coenzyme reductase (*mcrA*) and the alpha subunit of dissimilatory sulfite reductase (*dsrA*). The *mcrA* gene catalyzes the reduction of a methyl group to CH_4 (Thauer, 1998), and is possessed by all known methanogens thereby making it ideal for quantifying methanogen abundance (Luton et al., 2002; Earl et al., 2003; Castro et al., 2004). The *dsrA* gene catalyzes the final step in sulfate respiration, and its ubiquity in sulfate-reducing bacteria makes it powerful at assessing their abundance (Wagner et al., 1998; Klein et al., 2001; Zverlov et al., 2005). Although the number of genes does not necessarily equate with number of cells or gene activity, qPCR of functional genes for particular guilds is a commonly used approach to estimate the abundance of a functional group and these gene abundances have been correlated with functional processes such as CH_4 production (e.g., Morris et al., 2015).

The *mcrA* and *dsrA* genes were amplified using a 20-µL qPCR reaction in a Mastercycler ep realplex^2 gradient S (Eppendorf, Hamburg, Germany), using SYBR Green as the reporter dye. Each reaction contained 1 µL of brackish or freshwater wetland DNA template and was conducted using the PerfeCTa SYBR Green FastMix (Quanta BioSciences). For the *mcrA* qPCR, primer details and thermocycling conditions in West et al. (2012) were replicated except that we employed a fluorescent detection step at 78 ºC for 20 seconds. For the *dsrA* qPCR primer, details and thermocycling conditions in Kondo et al. (2008) were replicated. Melting curves for both *mcrA* and *dsrA* were run to ensure absence of non-specific amplification. Amplification, fluorescence data collection, and initial data analysis were all performed by the Eppendorf realplex^2 software.

Standard qPCR curves for *mcrA* and *dsrA* were generated by pooling gel-extracted amplicons containing our qPCR primer sites from a subset of our non-tidal freshwater and tidal brackish wetland samples. We amplified *mcrA* using primers detailed in Luton et al. (2002) and thermocycling conditions in West et al. (2012), and *dsrA* by replicating primer details and thermocycling conditions in Kondo et al. (2008). After amplification, we used gel electrophoresis and an Invitrogen PureLink Quick Gel Extraction Kit (Invitrogen, Carlsbad, CA, USA) to isolate the *mcrA* and *dsrA* amplicons. Following clean-up, we quantified the purified amplicons using Invitrogen's Qubit technology. We then used serial ten-fold dilutions of these genes to generate standard curves for qPCR. Our detection limit for each gene was approximately 1000 copies per g of wet sediment. Samples below detection were assigned a value of 999 copies per g for further analysis. We ran triplicate analyses of all samples for both the *mcrA* and *dsrA* qPCR, the averages of which were used in summary statistics and analyses.

2.4 Statistical analyses

For the non-tidal freshwater (n = 18, 9 sites x 2 time periods) and tidal brackish wetland comparison (n = 10, 5 sites x 2 time periods), we analyzed how four factors influenced log-transformed CH_4 production rates using generalized linear models (GLM) and Akaike Information Criterion (AIC) based model selection. The four factors were: (1) ecosystem type (non-tidal freshwater or tidal brackish), (2) time period (June or August), (3) porewater acetate availability (nmol g^{-1} dry sediment), and

(4) total sulfate present (nmol g^{-1} dry sediment). As nitrate availability was extremely low in these ecosystems in comparison to total sulfate availability (i.e., ~5%), we did not include nitrate as a factor in the GLMs. AIC-based model selection identifies the most likely model given the data while penalizing for model complexity (i.e., the number of parameters). In our analysis, we corrected for small sample sizes (AIC_c; Burnham and Anderson 2002). The model with the lowest AICc value is considered the most likely, and all remaining models are compared relative to the most likely model using delta AIC_c (Δ_i). Models with a Δ_i less than or equal to 2 are considered to have substantial support, while models having a Δ_i greater than 7 have little support (Burnham and Anderson 2002). The relative strength of our candidate models was then evaluated with Akaike weights (ω_i), which indicate the probability of a model being the most likely model, given the data and the set of candidate models (Burnham and Anderson 2002). We considered 16 candidate models (all possible additive combinations of the four factors including the null model) using the methods described above. A subset of those models, excluding the null model (i.e., intercept only) and those with relatively low support ($\Delta_i > 4$), were then used to determine model-averaged parameter estimates and to estimate the relative importance of variables (Burnham and Anderson, 2002). To estimate the relative importance of predictor variable *x*, we used the sum of Akaike weights for models including variable *x* (the closer the sum is to 1, the more important the variable *x*); we only considered models where $\Delta_i < 4$ for this analysis (Burnham and Anderson, 2002).

To compare the abundance of methanogens and sulfate-reducing bacteria, we first used a chi-squared test for each gene to determine whether the presence/absence of *mcrA* or *dsrA* was independent of ecosystem type. We then used a non-parametric Kruskal-Wallis tests to determine whether the number of copies of *mcrA* or *dsrA* varied by ecosystem type. For all statistical analyses excluding AIC model selection, α was set 0.05.

For the seawater addition experiment, we conducted a paired *t*-test to determine whether CH_4 production rates in non-tidal freshwater wetland sediments were affected by being incubated anaerobically with brackish tidal water instead of freshwater from their respective wetlands. Pearson correlations were computed (Zar, 2010) to determine whether porewater acetate or total sulfate levels were related to CH_4 production rates during this experiment.

To determine whether adding organic matter affected CH_4 production rates, we first used an analysis of variance (ANOVA) with treatment (i.e., macrophyte species) as the factor of interest and non-tidal freshwater wetland as a blocking variable. Then we analyzed how three factors influenced the response of each sediment, or ΔCH_4 production (treatment–control), using additive GLMs. The three factors were: (1) macrophyte species added, (2) total acetate available in the porewater (nmol g^{-1} dry sediment), and (3) total amount of sulfate present (nmol g^{-1} dry sediment). A total of eight candidate models (all possible additive combinations of the three factors including the null model) were compared as described above. To determine whether macrophyte species stoichiometry influenced the response of methanogens to increased organic matter, linear regressions were computed for % C, % N, % P, C:N, and C:P (from Tiegs et al., 2013) against ΔCH_4 production. All

statistical analyses were conducted in the R software environment using the base and MuMIn packages (R Development Core Team, 2016).

3 Results

3.1 Non-tidal freshwater and tidal brackish wetland comparison

3.1.1 Water column and porewater chemistry

Water column and sediment porewater chemistry of the incubations varied by ecosystem type (Tables 1 & 2), and variation by ecosystem type tended to be greater than temporal variation. Total sulfate levels in non-tidal freshwater incubations (June: 84 ± 65; August: 48 ± 43 nmol gram^{-1} dry sediment; mean ± sd) were about two orders of magnitude lower than in tidal brackish incubations (June: 4300 ± 4300; August: 3500 ± 3700 nmol gram^{-1} dry sediment) and did not vary between time periods. In comparison to total sulfate levels, porewater nitrate availability was very low, with non-tidal freshwater wetlands (June: 1.5 ± 0.9; August: 1.8 ± 1.8 nmol gram^{-1} dry sediment) having relatively higher nitrate than the tidal brackish wetlands (June: 0.24 ± 0.51; August: 0.0092 ± 0.0025 nmol gram^{-1} dry sediment; Table 2). The total amount of acetate available in the non-tidal freshwater wetland incubations was similar in June (28 ± 22 nmol gram^{-1} dry sediment) and August (30 ± 17 nmol gram^{-1} dry sediment), while levels in the tidal brackish wetland incubations were generally higher and more variable especially in August (210 ± 260 nmol gram^{-1} dry sediment) than in June (130 ± 80 nmol gram^{-1} dry sediment).

3.1.2 CH_4 production

CH_4 production rates were higher in non-tidal freshwater wetlands than in tidal brackish wetlands and approximately an order of magnitude higher in both ecosystems in August compared to June (Fig. 3). Porewater acetate was positively associated with higher CH_4 production rates, while higher total sulfate availability was associated with lower CH_4 production rates (Table 3). The most likely model contained all four factors – ecosystem type, time period, acetate, and total sulfate (Table 3). Based upon model averaging of the top three models (Table 3), all four factors appeared to influence CH_4 production with the relative importance of these variables being 1.00 for ecosystem, 1.00 for porewater acetate, 0.87 for total sulfate availability, and 0.74 for time period.

3.1.3 Functional group abundances

Tidal brackish sediments tended to have higher abundances of sulfate-reducing bacteria when present, while non-tidal freshwater sediments were characterized by higher numbers of methanogens. In the tidal brackish wetlands, three out of ten samples were below the detection limit for the *dsrA* gene, our proxy for sulfate-reducing bacteria abundance, but we detected this gene in all nine non-tidal freshwater wetland composite samples. The presence or absence of the *dsrA* gene was independent of ecosystem type ($\chi^2 = 3.21$, df = 1, $P = 0.07$). Tidal brackish sediments (n = 10) and non-tidal freshwater wetland

sediments (n = 9) had 3.52 ± 5.39 x 10^5 and 5.20 ± 5.08 x 10^4 copies of *dsrA* per gram of wet sediment, respectively. Due to high variability, the number of copies of *dsrA* did not differ significantly by ecosystem (Kruskal-Wallis: H = 1.31, df = 1, P = 0.25). In contrast, we detected the *mcrA* gene, our proxy for methanogen abundance, in only two out of ten tidal brackish samples, but in all nine non-tidal freshwater wetland samples. The presence or absence of the *mcrA* gene was dependent on ecosystem type (χ^2 = 12.44, df = 1, P = 0.0004). Tidal brackish samples had 2.14 ± 5.78 x 10^4 copies of the *mcrA* per gram of wet sediment, while non-tidal freshwater wetlands had 1.84 ± 1.25 x 10^5 copies of *mcrA* per gram of wet sediment. Methanogen abundance therefore differed significantly between ecosystem types (Kruskal-Wallis: H = 11.24, df = 1, P = 0.0008)

3.2 Seawater addition experiment

Incubating non-tidal freshwater wetland soils with brackish water did not affect CH_4 production rates (Fig. 4). Even though total sulfate levels increased from 63 ± 37 to 5400 ± 400 nmol $gram^{-1}$ dry sediment with the addition of tidal brackish water, CH_4 production rates did not differ between treatment and control incubations (paired t-test: t = 0.44, df = 4, P = 0.68). However, CH_4 production rates were significantly correlated with porewater acetate levels (r = 0.88, t = 5.18, df = 8, P = 0.0008), but not with total sulfate levels (r = 0.09, t = 0.24, df = 8, P = 0.81). The non-tidal freshwater wetland sediments used in this seawater addition experiment (n = 5) had about an order of magnitude higher number of copies of *mcrA* (3.12 ± 4.40 x 10^5) than *dsrA* (5.32 ± 6.33 x 10^4) per gram of wet sediment.

3.3 Increased organic matter simulation

The organic matter treatments significantly influenced CH_4 production rates ($F_{4, 16}$ = 4.48, P = 0.01), but this effect varied with macrophyte species (Fig. 5). Adding buckbean and marestail had little effect on CH_4 production, while the lily and horsetail treatments generally increased methanogen activity (Fig. 5). The most likely model for predicting ΔCH_4 production (treatment – control) included acetate availability, which had a negative effect on the response (Table 4). The next best models included porewater acetate and species (Model 2) or porewater acetate and total sulfate availability (Model 3), which had a positive effect on the response (Table 4). Models 1–4 (Table 4) were averaged to determine parameter estimates with the relative importance of the variables being 0.88 for porewater acetate, 0.33 for macrophyte species, and 0.15 for total sulfate availability. Using the model-averaged parameters, our predictions of the response of CH_4 production rates to increased substrate availability closely followed the observed results (Fig. 6). Finally, macrophyte species stoichiometry (i.e., % C, % N, % P, C:N, and C:P) had no effect on ΔCH_4 production ($r^2 < 0.08$, $P > 0.24$ for all regressions).

4 Discussion

To begin to understand likely responses of wetlands to global change processes, we conducted a space-for-time substitution of how seawater intrusion might affect CH_4 production in freshwater wetlands by comparing them to brackish systems. We found that CH_4 production was lower in tidal brackish than in non-tidal freshwater wetlands, likely due to differences in availability of alternative electron acceptors (i.e., higher sulfate levels in the tidal brackish) and in microbial communities (i.e., lower methanogen abundances in the tidal brackish). Experimental addition of seawater in non-tidal freshwater sediments (~14 days), however, did not influence CH_4 production rates. In contrast, higher organic matter availability enhanced CH_4 production rates in 75% of incubations, but this response depended on the amount of substrate already available and the macrophyte species added, with half of the species treatments having no significant effect. Because acetate and sulfate availability had contrasting effects depending on the experiment (i.e., freshwater/brackish comparison vs. increased organic matter), these results indicate that we do not have a sufficient mechanistic understanding of how changes in electron donors and electron acceptors will interact to ultimately influence CH_4 production. Future studies should consider the possible interaction of global change mechanisms, such as sea-level rise and CO_2 fertilization/longer growing seasons, which will likely alter the availability of electron acceptors and electron donors, thereby influencing CH_4 production (Fig. 1).

4.1 Non-tidal freshwater and tidal brackish wetland comparison

CH_4 production rates in tidal brackish wetlands were substantially lower than those of non-tidal freshwater wetlands, as predicted. Many studies have attributed the decrease in wetland CH_4 emissions along increasing salinity and sulfate concentrations to sulfate-reducing bacteria outcompeting methanogens for substrates (DeLaune et al., 1983; Bartlett et al., 1987; Magenheimer et al., 1996; Poffenbarger et al., 2011), but none of these studies directly assessed whether lower CH_4 emissions resulted from reduced CH_4 production or higher CH_4 oxidation. Two recent studies documented lower CH_4 production with elevated salinity (Chambers et al., 2013; Neubauer et al., 2013), and attempted to link C mineralization rates to extracellular enzymes, but microbial communities were not quantified. In comparison, our study quantified CH_4 production along a similar spatial gradient and directly linked lower CH_4 production to higher sulfate availability and indirectly to relative abundance of functional microbial guilds. The presence of alternative electron acceptors such as sulfate likely indicates that methanogens have to compete for organic substrates with sulfate-reducing bacteria (Oremland and Polcin, 1982; Lovley and Klug, 1986; Achtnich et al., 1995). Our study also demonstrates that tidal brackish sediments tended to have generally higher sulfate-reducing bacteria (*dsrA*) abundances when present, but significantly lower levels of methanogens (*mcrA*) than non-tidal freshwater sediments. Although we did not include microbial data in the model selection due to sample size limitations, we hypothesize that microbial community differences could help to explain why ecosystem type (freshwater vs. brackish) was an important factor during model selection. Collectively, these results along with higher sulfate availability in tidal brackish

wetlands (and sulfate's importance in our model selection analysis) suggest that shifts in the relative abundance of functional microbial guilds between tidal brackish and non-tidal freshwater wetlands contribute to differences in CH_4 production between these ecosystems.

The difference between brackish and freshwater wetland CH_4 production could also be shaped by other ecosystem factors such as salinity and salinity-induced cation exchange. Because salinity and sulfate availability are often correlated, it can be difficult to disentangle these two factors; Chambers et al. (2011) isolated their effects in a laboratory manipulation and found that seawater (sulfate) had a more dramatic and longer lasting effect on CH_4 production than saltwater (NaCl). Nevertheless, salinity often places additional stress on organisms, such that saltwater intrusion alters microbial and plant communities (Herbert et al., 2015). Additionally, saltwater intrusion can influence cation exchange in the sediments, such that calcium is mobilized, which can co-precipitate with phosphate, and ammonium is released, all of which can shift a wetland towards P rather than N limitation (Herbert et al., 2015; van Dijk et al., 2015). Although we did not directly measure these effects of salinity and therefore cannot rule them out, we hypothesize that sulfate availability and differences in functional microbial guilds are primarily responsible for differences in CH_4 production rather than salinity and salinity-induced cation exchange. Our hypothesis relies on three observations: (1) N and P availability were extremely low in both freshwater and brackish ecosystems (DIN: < 25 µg N L^{-1}, SRP: < 15 µg P L^{-1}) and therefore different sediment cation exchange capacities were unlikely to change the N and P limitation of these wetlands, (2) salinity tended to be consistently low in freshwater wetlands, but CH_4 production was still negatively correlated with sulfate availability, and (3) sulfate availability was an important factor in ecosystem comparison model selection, and was the only factor where a direct mechanistic link can be made to the differences in CH_4 production between freshwater and brackish ecosystems (i.e., acetate availability was higher in brackish wetlands and therefore one might expect higher CH_4 production).

In addition to the influences of microbial communities and alternative electron acceptors on CH_4 production, acetate availability appeared to be an important factor. Substrate availability regulates CH_4 production (Whalen, 2005), and acetate is one of the major precursors for methanogenesis (Conrad, 1999) as it can be a direct (acetoclastic) or an indirect (hydrogentrophic) substrate for methanogens after further fermentation. Although the importance of acetate as a factor in our experiments suggests that acetoclastic methanogenesis may be prevalent in the CRD, we cannot rule out the potential of hydrogenotrophic methanogenesis, which is thought to be the primary pathway in other Alaskan wetlands (Hines et al., 2001). According to Hines et al. (2008), acetate tended to accumulate in Alaskan peat rather than be converted to CH_4 possibly due to homoacetogenic bacteria (i.e., those that make acetate) being able to outcompete methanogens for CO_2 and H_2 in colder temperatures and the general lack of acetoclastic methanogens. In contrast, CH_4 production in CRD wetlands was tightly related to acetate availability in the ecosystem comparison as well as in both simulations. Despite the differences between these Alaskan wetlands (CRD sediment is more similar to clay than to peat; see SOM % in Table 2), CRD freshwater wetlands

exhibited similar CH_4 production rates to those conducted during August 2001 by Hines et al. (2008), which ranged from about 10 to 500 nmol g^{-1} dry peat day^{-1}. Because CH_4 production rates in Alaskan peat tended to increase with higher proportions of vascular plant cover (Hines et al., 2008) and the fermentation of this plant matter facilitates the production of acetate, it is possible that the role of the acetoclastic pathway may grow more important in northern wetlands in the future as vascular plant growth increases (Klady et al., 2011).

CH_4 production rates often vary seasonally as a function of temperature, but we observed August rates that were an order of magnitude higher than those conducted in June despite these incubations being conducted at the same temperature. Other factors affecting CH_4 production that could vary seasonally include (1) availability of organic matter such as acetate for CH_4 production (Whiting and Chanton, 1993; Walter et al., 2001), (2) availability of alternative electron acceptors including sulfate (Sinke et al., 1992), (3) microbial population densities (Yannarell and Triplett, 2005), or (4) the pathway by which CH_4 is produced (Avery et al., 1999). In our study, we did not observe large seasonal differences in porewater acetate or sulfate availability in CRD wetlands, but we did not assess seasonal variation in the abundances of methanogens and sulfate-reducing bacteria, their per-cell activity rates, or availability of H_2 or methanogenic substrates other than acetate. Therefore, it is possible that the observed seasonal differences in CH_4 production rates were the result of microbial community shifts, decreased per-cell activity of methanogens in June, greater CH_4 produced from the hydrogenotrophic pathway during August as acetate levels did not change, or some combination of these potential explanations. Additionally, we acknowledge that the porewater acetate level we measured is an indicator of the balance between acetogenesis and acetate consumption, so it is possible that acetogenesis rates increased during August and the acetoclastic pathway of methanogenesis correspondingly increased such that acetate availability appeared to be similar during these two months. Although we did not collect the data that satisfactorily explain these intriguing seasonal differences, we hypothesize that CH_4 production rates vary in accordance with macrophyte phenology in these ecosystems, which clearly affects both the availability of electron donors and microbial processing rates (e.g., Eviner and Chapin, 2003). We think the following seasonal trajectory is possible: (1) In early growing season, CH_4 production is low, but steeply increases at peak growing season as more labile plant exudates are produced, and (2) The end of the growing season results in plant senescence, increased organic matter availability as plants decompose, and reduced oxygen levels, which then results in higher CH_4 production until colder temperatures start to decelerate microbial processing. All of these conditions could lead to seasonal succession in microbial communities and their activity rates. Future studies should seek to explain the mechanism behind seasonal differences in CH_4 production that are independent of temperature.

4.2 Seawater addition experiment

Despite our finding that CH_4 production rates were significantly lower in tidal brackish wetlands sites, adding seawater to non-tidal freshwater sediments surprisingly did not affect CH_4 production rates. We acknowledge that our experiment simulated

short-term consequences of seawater intrusion such as increased sulfate availability and the addition of other marine nutrients and microbial communities, but we were not simulating longer term changes such as differences in plant communities and production that may result from increased salinity (Neubauer 2013; Hopfensperger et al., 2014; Herbert et al., 2015). Nevertheless, many other short-term studies conducting similar seawater addition experiments have observed a decrease in CH_4 production rates with elevated salinity (DeLaune et al., 1983; Chambers et al., 2011; Marton et al., 2012; Chambers et al., 2013; Neubauer et al., 2013; van Dijk et al., 2015). In many of these studies, however, sulfate availability was much higher. For example, DeLaune et al. (1983) found that CH_4 production was inhibited with the addition of ~10 mM sulfate, which is higher than the sulfate concentration (~6 mM) used in this study. Chambers et al. (2011) observed a reduction in the treatments where sulfate concentrations were about 130 and 320 µmol per g^{-1} of dry sediment, which is over one order of magnitude larger than our seawater addition experiment (5 µmol g^{-1} of dry sediment). Additionally, the majority of all these experiments were conducted at 25–30 °C, or almost double the temperature used in this study (14 °C), which could increase the rates at which microbial communities and their activities respond. It is therefore likely that the external environmental conditions imposed, such as the temperature, salinity, or sulfate availability used in a seawater addition experiment, can influence the results.

In addition to environmental conditions, initial factors such as soil characteristics or site properties may mediate how methanogens respond to seawater addition experiments (Neubauer et al., 2013). For example, van Dijk et al. (2015) found that elevated salinity decreases CH_4 production in peat but not in clay, and the sediment of the CRD wetlands is claylike in nature. Additionally, in some of these experiments, the sediments prior to incubation had been exposed to higher levels of sulfate (e.g., brackish sediments used by DeLaune et al. 1983), and microbial communities therefore could have been more likely to respond with higher rates of sulfate reduction, thereby increase competition for organic substrates. In contrast, the freshwater sediments used in this simulation had lower sulfate availability, and the sulfate-reducing bacteria abundances were an order of magnitude lower than methanogens. In some cases, however, sulfate reduction can increase without a corresponding decrease in CH_4 production (Hopfensperger et al., 2014), especially if seawater intrusion increases both sulfate and organic matter availability (Weston et al., 2011).

Seawater intrusion could therefore affect both availability of alternative electron acceptors and organic matter, but their contrasting effects on CH_4 production are mediated by microbial communities and processes. Although the presence of sulfate-reducing bacteria was detectable in the sediments used in this simulation, we do not know whether these taxa were active or dormant. In fact, dormant taxa can account for almost 40% of taxon richness in nutrient-poor systems (Jones and Lennon, 2010), such as the CRD freshwater wetlands. Additionally, we conducted 14-day incubations, which may have been too short to allow for shifts in the relative abundance of sediment microbial populations (Hoehler and Jørgensen, 2013). For example, Edmonds et al. (2009) found no changes in microbial community composition of bacteria or archaea after sediment

cores had been exposed to seawater for 35 days. We therefore hypothesize that the reason that CH_4 production in freshwater sediments did not respond to the seawater addition experiment is a combination of environmental conditions, initial sediment factors, and a lag in response time from the microbial communities.

4.3 Increased organic matter simulation

Higher availability of organic matter generally increased CH_4 production rates, but this effect varied with the species of macrophyte added to the incubations. Differences in litter quality is known to influence methanogen communities and CH_4 production (Yavitt et al., 1990; 2000; Valentine et al., 1994). For example, West et al. (2012) found that adding algal carbon significantly enhanced CH_4 production relative to terrestrial carbon. Although aquatic macrophyte carbon may be of lower quality than that of algae, aquatic macrophytes are generally more labile than terrestrial plants (Schlickeisen et al., 2003). For example, Tiegs et al. (2013) found that terrestrial plants decomposed more slowly than aquatic macrophytes in CRD wetlands. Additionally, Tiegs et al. (2013) conducted a decomposition assay of all the macrophyte species used in this study, as a way of assessing litter quality, and found that buckbean and lily leaves decomposed at about the same rate, but both were faster than marestail and horsetail. The rate of decomposition of different plant species was correlated with phosphorus content, and therefore indicative of litter quality differences (Tiegs et al., 2013). However, our CH_4 production response did not follow the decomposition pattern documented by Tiegs et al. (2013); we observed higher CH_4 production for the lily and horsetail treatment relative to the control, but not for buckbean and marestail. We also did not find that the CH_4 production response to organic matter treatment varied by % C, % N, % P, C:N, C:P, or any other measure of litter quality assessed by Tiegs et al. (2013).

Other measures of litter quality beyond elemental composition could explain differences in the methanogen response. West et al. (2015), for example, found that higher lipid content of phytoplankton enhanced CH_4 production rates. Alternatively, certain properties may influence the fermentative microbial communities associated with vegetation during decomposition (Boon et al., 1996), which are responsible for providing methanogenic substrates. For example, in a survey of 209 plants, Bishop and MacDonald (1951) reported that buckbean was one of the 10 most active species for antibacterial substances, while horsetail did not possess such properties. Specifically, buckbean extracts include aucubin, a defensive compound that can inhibit many strains of anaerobic bacteria (Weckesser et al., 2007). Marestail also contains aucubin as well as a verbascoside, another antimicrobial compound (Damtoft et al., 1994). In contrast, the only part of lily linked to potential antimicrobial properties is the rhizomes, which have been used in folk medicine (Padgett, 2007) and are more likely to require defensive compounds because of competition with the sediment microbial community than the floating leaves we used for this experiment. Therefore, we hypothesize that CH_4 production varied as a function of a different measure of litter quality than previously put forward (e.g., C:N:P, percent lignin, or lipid content), whereby the negative effects of the antimicrobial

properties of buckbean and marestail on the fermentative bacteria superseded the positive effect of increasing the amount of organic matter. We suggest that this hypothesis is worthy of further examination.

Many other studies have documented that CH_4 production is enhanced by the addition of direct substrates such as acetate and H_2 (Williams and Crawford, 1984; Bachoon and Jones, 1992; Amaral and Knowles, 1994; Coles and Yavitt, 2002; Yavitt and Seidman-Zager, 2006), or the addition of indirect substrates such as dextrose and glucose (DeLaune et al., 1983; Williams and Crawford, 1984; Coles and Yavitt, 2002), which would need to be broken down by fermentative bacteria before methanogens could utilize them. Fewer studies have examined the effects of more biologically realistic, indirect substrates such as plant or algal matter on CH_4 production incubations (but see Valentine et al., 1994; West et al., 2012; 2015). However, two studies involving larger scale plots with elevated CO_2 levels exhibited greater photosynthetic rates and greater CH_4 emissions (Megonigal and Schlesinger, 1997; Vann and Megonigal, 2003). Although Vann and Megonigal (2003) observed enhanced plant biomass that was strongly correlated with CH_4 emissions, Megonigal and Schlesinger (1997) did not see increased biomass and therefore hypothesized that lower transpiration rates, not increased substrate availability, led to higher CH_4 emissions by increasing flooding duration and stimulating anaerobic processes. In our study, increased substrate availability is likely the mechanism behind increased CH_4 production because our smaller scale simulation did not alter flooding duration, anaerobic conditions, or the physical structures by which plants can act as conduits for gas exchange (i.e., aerenchyma). Interestingly, the amount of acetate already available in the sediment appeared to moderate the methanogen response to enhanced substrate availability. The negative relationship between ΔCH_4 production and porewater acetate concentration suggests that methanogenic substrate concentrations can become saturated, which is expected from traditional Michaelis-Menten enzyme kinetics.

Another indication of substrate limitation is the positive relationship between the methanogenic response to added organic matter and the total amount of sulfate available in the incubation. This alternative electron acceptor provides more energy than either methanogenic pathway (acetoclastic or hydrogenotrophic) when coupled to the oxidation of organic matter (Stumm and Morgan, 1996; Schlesinger and Bernhardt, 2013). For example, Westermann and Ahring (1987) found that inhibiting sulfate reduction stimulated CH_4 production in an alder swamp, suggesting that methanogens and sulfate-reducing bacteria compete for common substrates. Sulfate availability, therefore, may signal strength of competition for electron donors (organic matter) that methanogens must overcome to produce CH_4. The higher the competition, the more likely that methanogens respond positively to the addition of organic matter. The response of methanogens to increased substrate availability, therefore, is likely regulated by the quality of the substrate (e.g., C:P, lipid content, or antimicrobial compounds), strength of competition for substrate (e.g., availability of alternative electron acceptors, microbial community assemblages, or per-cell activity rates), and whether substrate availability is limiting or saturated in the environment. Although total sulfate

availability played a less significant role than acetate and macrophyte species, the model using averaged estimates from all three parameters allowed us to accurately predict the response in CH_4 production for this experiment.

5 Conclusions

Our study demonstrates that potential interactions between elements of global change, specifically seawater intrusion and increased organic matter from longer growing seasons and CO_2 fertilization, could have competing effects on CH_4 production from coastal wetlands (Fig. 1). Determining the timescale required for processes at the microbial scale to shift towards sulfate reduction is challenging, and the magnitude of seawater intrusion needed to induce this shift is currently unclear. Microbial community shifts can occur over longer timescales than several months, and CH_4 production can be more affected by long-term salinization (~ 3.5 years) than 2-day salinity pulses (Neubauer et al., 2013). As others have noted, the global carbon cycle is inextricably linked to other elemental cycles (i.e., sulfur) by processes taking place at the microbial scale (Schimel, 2004; Burgin et al., 2011). In addition, the potential effects of seawater intrusion are not limited to CH_4 production alone. Salinization also reduces aerobic and anaerobic methane oxidation, with aerobic organisms being particularly sensitive to salinity (Dalal et al., 2008; Herbert et al., 2015). Furthermore, the effects of sulfate availability on the CH_4 cycle extend beyond sea-level rise to other aspects of global change such as road salts and agricultural land use (Helton et al., 2014; Herbert et al., 2015).

In contrast to sea-level rise and increased sulfate availability, longer growing seasons and CO_2 fertilization will likely enhance carbon substrate supply and in turn CH_4 production. Our study demonstrates that the effect of increased organic matter depends on plant species, the availability of other methanogenic substrates, and the presence of alternative electron acceptors. It is possible that longer growing seasons and CO_2 fertilization could reduce competition between methanogens and other microbial communities by providing more substrates, as we saw in freshwater wetlands with higher sulfate concentrations, thereby superseding the effect of seawater intrusion. Additionally, the CO_2 fertilization effect could increase organic matter accretion of marsh plants, which could physically counteract sea-level rise by raising marsh elevation (Langley et al., 2009). Future studies should consider how the interaction of sea-level rise, increased organic matter, and warming will affect both the microbial and ecosystem processes of the global methane cycle. This intersection of global change processes will be particularly important for projecting the future CH_4 budgets of coastal wetland ecosystems.

6 Data availability

The data will be freely accessible through the international repository, Knowledge Network for Biocomplexity (KNB) at: https://knb.ecoinformatics.org/#view/doi:10.5063/F1028PF8.

Author contributions. CV designed the study as sparked from discussions with SEJ. CV and JAH conducted the fieldwork and laboratory analyses. WEW played a key role in methodology and analyzing methane samples with the GC. SEJ and GAL played advisory roles in shaping this research. CV prepared the manuscript with contributions from all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgments. We thank the Cordova Ranger District of the USDA Forest Service for providing field and logistical support, particularly Deyna Kuntzsch, Andrew Morin, Sean Meade, Luca Adelfio, and Ken Hodges, without whom this work on the Copper River Delta would not have been possible. We also thank Gordie Reeves of the Pacific Northwest Research Station for his leadership and direction in the extensive research being conducted on the Copper River Delta. Mike Brueseke, Melanie Runkle, and Josephine Chau assisted with DOC and SOM analyses. The Center for Environmental Science and Technology at UND provided instrumentation and analytical assistance for the chemical analyses. Funding was provided by the USDA Forest Service, the Pacific Northwest Research Station, the National Fish and Wildlife Foundation, the University of Notre Dame, and the National Science Foundation Graduate Research Fellowship Program. We also thank members of the Jones laboratory and the Lamberti laboratory at UND for their feedback on the manuscript. We acknowledge Drs. Mary Scranton, Scott Neubauer, and Marcelo Ardón, as well as an anonymous reviewer who put tremendous effort into reviewing this manuscript and whose comments and suggestions greatly improved its quality.

References

Achtnich, C., Bak, F. and Conrad, R.: Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil, Biol. Fertil. Soils, 19(1), 65–72, 1995.

Amaral, J. A. and Knowles, R.: Methane metabolism in a temperate swamp, Appl. Environ. Microbiol., 60(11), 3945–3951, 1994.

Avery, G. B., Shannon, R. D., White, J. R., Martens, C. S. and Alperin, M. J.: Effect of seasonal changes in the pathways of methanogenesis on the δ13C values of pore water methane in a Michigan peatland, Glob. Biogeochem. Cycles, 13(2), 475–484, 1999.

Bachoon, D. and Jones, R. D.: Potential rates of methanogenesis in sawgrass marshes with peat and marl soils in the everglades, Soil Biol. Biochem., 24(1), 21–27, 1992.

Bartlett, K. B., Bartlett, D. S., Harriss, R. C. and Sebacher, D. I.: Methane emissions along a salt marsh salinity gradient, Biogeochemistry, 4(3), 183–202, 1987.

Bishop, C. J. and MacDonald, R. E.: A survey of higher plants for antibacterial substances, Can. J. Bot., 29(3), 260–269, 1951.

Boon, P., Virtue, P. and Nichols, P.: Microbial consortia in wetland sediments: a biomarker analysis of the effect of hydrological regime, vegetation and season on benthic microbes, Mar. Freshw. Res., 47(1), 27–41, 1996.

Bryant, M. D.: The Copper River Delta pulse study: an interdisciplinary survey of aquatic habitats, General Technical Report, U.S.D.A., Forest Service, Pacific Northwest Research Station, Portland, Oregon., 1991.

Burgin, A. J., Yang, W. H., Hamilton, S. K. and Silver, W. L.: Beyond carbon and nitrogen: how the microbial energy economy couples elemental cycles in diverse ecosystems, Front. Ecol. Environ., 9(1), 44–52, 2011.

Burnham, K. P. and Anderson, D. R.: Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach, 2nd ed., Springer-Verlag, Inc., New York, NY., 2002.

Castro, H., Ogram, A. and Reddy, K. R.: Phylogenetic characterization of methanogenic assemblages in eutrophic and oligotrophic areas of the Florida Everglades, Appl. Environ. Microbiol., 70(11), 6559–6568, 2004.

Chambers, L. G., Reddy, K. R. and Osborne, T. Z.: Short-term response of carbon cycling to salinity pulses in a freshwater wetland, Soil Sci. Soc. Am. J., 75, 2000–2007, 2011.

Chambers, L. G., Osborne, T. Z. and Reddy, K. R.: Effect of salinity-altering pulsing events on soil organic carbon loss along an intertidal wetlands gradient: a laboratory experiment, Biogeochemistry, 115, 363–383, 2013.

Coles, J. R. P. and Yavitt, J. B.: Control of methane metabolism in a forested northern wetland, New York State, by aeration, substrates, and peat size fractions, Geomicrobiol. J., 19(3), 293–315, 2002.

Conrad, R.: Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments, FEMS Microbiol. Ecol., 28(3), 193–202, 1999.

Dalal, R., Allen, D., Livesley, S. and Richards, G.: Magnitude and biophysical regulators of methane emission and consumption in the Australian agricultural, forest, and submerged landscapes: a review. Plant Soil, 309: 43–76, 2008.

Damtoft, S., Rosendal Jensen, S., Thorsen, J., Mølgard, P. and Erik Olsen, C.: Iridoids and verbascoside in Callitrichaceae, Hippuridaceae and Lentibulariaceae, Phytochemistry, 36(4), 927–929, 1994.

DeLaune, R. D., Smith, C. J. and Patrick, W. H.: Methane release from Gulf coast wetlands, Tellus B, 35B(1), 8–15, 1983.

Earl, J., Hall, G., Pickup, R. W., Ritchie, D. A. and Edwards, C.: Analysis of methanogen diversity in a hypereutrophic lake using PCR-RFLP analysis of mcr sequences, Microb. Ecol., 46(2), 270–278, 2003.

Edmonds, J. W., Weston, N. B., Joye, S. B., Mou, X. and Moran, M. A.: Microbial community response to seawater amendment in low-salinity tidal sediments, Microb. Ecol., 58(3), 558–568, 2009.

Freymueller, J. T., Woodard, H., Cohen, S. C., Cross, R., Elliott, J., Larsen, C. F., Hreinsdóttir, S. and Zweck, C.: Active deformation processes in Alaska, based on 15 Years of GPS measurements, in Active Tectonics and Seismic Potential of Alaska, edited by J. T. Freymueller, P. J. Haeussler, R. L. Wesson, and G. Ekström, pp. 1–42, American Geophysical Union, 2008.

Eviner, V. T. and Chapin, F. S., III: Functional matrix: a conceptual framework for predicting multiple plant effects on ecosystem processes, Annu. Rev. Ecol. Evol. Syst., 34, 455–485, 2003.

Gedney, N., Cox, P. M. and Huntingford, C.: Climate feedback from wetland methane emissions, Geophys. Res. Lett., 31(20), 1–4, 2004.

Helton, A. M., Bernhardt, E. S. and Fedders, A.: Biogeochemical regime shifts in coastal landscapes: the contrasting effects of saltwater incursion and agricultural pollution on greenhouse gas emissions from a freshwater wetland, Biogeochemistry, 120, 133–147, 2014.

Herbert, E. R., Boon, P., Burgin, A. J., Neubauer, S. C., Franklin, R. B., Ardón, M., Hopfensperger, K. N., Lamers, L. P. M. and Gell, P.: The global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands, Ecosphere, 6(10), 206, 2015.

Hines, M. E., Duddleston, K. N. and Kiene, R. P.: Carbon flow to acetate and C1 compounds in northern wetlands, Geophys. Res. Lett., 28(22), 4251–4254, 2001.

Hines, M. E., Duddleston, K. N., Rooney-Varga, J. N., Fields, D. and Chanton, J. P.: Uncoupling of acetate degradation from methane formation in Alaskan wetlands: Connections to vegetation distribution, Glob. Biogeochem. Cycles, 22(2), 2008.
Hoehler, T. M. and Jørgensen, B. B.: Microbial life under extreme energy limitation, Nat. Rev. Microbiol., 11(2), 83–94, 2013.

Hopfensperger, K. N., Burgin, A. J., Schoepfer, V. A. and Helton, A. M.: Impacts of saltwater incursion on plant communities, anaerobic microbial metabolism, and resulting relationships in a restored freshwater wetland, Ecosystems, 17, 792–807, 2014.

Jones, S. E. and Lennon, J. T.: Dormancy contributes to the maintenance of microbial diversity, Proc. Natl. Acad. Sci., 107(13), 5881–5886, 2010.

Kirschke, S., Bousquet, P., Ciais, P., Saunois, M., Canadell, J. G., Dlugokencky, E. J., Bergamaschi, P., Bergmann, D., Blake, D. R., Bruhwiler, L., Cameron-Smith, P., Castaldi, S., Chevallier, F., Feng, L., Fraser, A., Heimann, M., Hodson, E. L., Houweling, S., Josse, B., Fraser, P. J., Krummel, P. B., Lamarque, J.-F., Langenfelds, R. L., Le Quéré, C., Naik, V., O'Doherty, S., Palmer, P. I., Pison, I., Plummer, D., Poulter, B., Prinn, R. G., Rigby, M., Ringeval, B., Santini, M., Schmidt, M., Shindell, D. T., Simpson, I. J., Spahni, R., Steele, L. P., Strode, S. A., Sudo, K., Szopa, S., van der Werf, G. R., Voulgarakis, A., van Weele, M., Weiss, R. F., Williams, J. E. and Zeng, G.: Three decades of global methane sources and sinks, Nat. Geosci., 6(10), 813–823, 2013.

Klady, R. A., Henry, G. H. R. and Lemay, V.: Changes in high arctic tundra plant reproduction in response to long-term experimental warming, Glob. Change Biol., 17(4), 1611–1624, 2011.

Klein, M., Friedrich, M., Roger, A. J., Hugenholtz, P., Fishbain, S., Abicht, H., Blackall, L. L., Stahl, D. A. and Wagner, M.: Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-reducing prokaryotes, J. Bacteriol., 183(20), 6028–6035, 2001.

Klinger, L. F., Zimmerman, P. R., Greenberg, J. P., Heidt, L. E. and Guenther, A. B.: Carbon trace gas fluxes along a successional gradient in the Hudson Bay lowland, J. Geophys. Res. Atmospheres, 99(D1), 1469–1494, 1994.

Kondo, R., Shigematsu, K. and Butani, J.: Rapid enumeration of sulphate-reducing bacteria from aquatic environments using real-time PCR, Plankton Benthos Res., 3(3), 180–183, 2008.

Langley, J. A., McKee, K. L., Cahoon, D. R., Cherry, J. A. and Megonigal, J. P.: Elevated CO2 stimulates marsh elevation gain, counterbalancing sea-level rise, Proc. Natl. Acad. Sci., 106(15), 6182–6186, 2009.

Lofton, D. D., Whalen, S. C. and Hershey, A. E.: Effect of temperature on methane dynamics and evaluation of methane oxidation kinetics in shallow Arctic Alaskan lakes, Hydrobiologia, 721(1), 209–222, 2014.

Lovley, D. R. and Klug, M. J.: Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations, Appl. Environ. Microbiol., 45(1), 187–192, 1983.

Lovley, D. R. and Klug, M. J.: Model for the distribution of sulfate reduction and methanogenesis in freshwater sediments, Geochim. Cosmochim. Acta, 50(1), 11–18, 1986.

Lovley, D. R. and Phillips, E. J. P.: Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments, Appl. Environ. Microbiol., 53(11), 2636–2641, 1987.

Luton, P. E., Wayne, J. M., Sharp, R. J. and Riley, P. W.: The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill, Microbiology, 148(11), 3521–3530, 2002.

Magenheimer, J. F., Moore, T. R., Chmura, G. L. and Daoust, R. J.: Methane and carbon dioxide flux from a macrotidal salt marsh, Bay of Fundy, New Brunswick, Estuaries, 19(1), 139–145, 1996.

Marton, J. M., Herbert, E. R. and Craft, C. B.: Effects of salinity on denitrification and greenhouse gas production from laboratory-incubated tidal forest soils, Wetlands, 32, 347–357, 2012.

Matthews, H. D.: Implications of CO2 fertilization for future climate change in a coupled climate–carbon model, Glob. Change Biol., 13(5), 1068–1078, 2007.

McGuire, A. D., Anderson, L. G., Christensen, T. R., Dallimore, S., Guo, L., Hayes, D. J., Heimann, M., Lorenson, T. D., Macdonald, R. W. and Roulet, N.: Sensitivity of the carbon cycle in the Arctic to climate change, Ecol. Monogr., 79(4), 523–555, 2009.

Megonigal, J. P. and Schlesinger, W. H.: Enhanced CH_4 emissions from a wetland soil exposed to elevated CO_2, Biogeochemistry 37(1), 77–88, 1997.

Moore, T. R. and Dalva, M.: The influence of temperature and water table position on carbon dioxide and methane emissions from laboratory columns of peatland soils, J. Soil Sci., 44(4), 651–664, 1993.

Morris, R. I., Tale, V. P, Mathai, P. P, Zitomer, D. H., and Maki J. S.: mcrA gene abundance correlates with hydrogenotrophic methane production rates in full-scale anaerobic waste treatment systems, Lett. Appl. Microbiol., 62(2), 111–118, 2015.

Neubauer, S. C.: Ecosystem responses of a tidal freshwater marsh experiencing saltwater intrusion and altered hydrology, Estuaries Coasts, 36: 491–507, 2013.

Neubauer, S. C., Franklin, R. B. and Berrier, D. J.: Saltwater intrusion into tidal freshwater marshes alters the biogeochemical processing of organic carbon, Biogeosciences, 10, 8171–8183, 2013.

Neubauer, S. C. and Megonigal, J. P.: Moving beyond global warming potentials to quantify the climatic role of ecosystems, Ecosystems, 18, 1000–1013, 2015.

Oremland, R. S. and Polcin, S.: Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments, Appl. Environ. Microbiol., 44(6), 1270–1276, 1982.

Padgett, D. J.: A monograph of Nuphar (Nymphaeaceae), Rhodora, 109(937), 1–95, 2007.

Poffenbarger, H. J., Needelman, B. A. and Megonigal, J. P.: Salinity influence on methane emissions from tidal marshes, Wetlands, 31(5), 831–842, 2011.

Ringeval, B., Friedlingstein, P., Koven, C., Ciais, P., de Noblet-Ducoudré, N., Decharme, B. and Cadule, P.: Climate-CH4 feedback from wetlands and its interaction with the climate-CO2 feedback, Biogeosciences, 8(8), 2137–2157, 2011.

Schimel, J.: Playing scales in the methane cycle: From microbial ecology to the globe, Proc. Natl. Acad. Sci. U. S. A., 101(34), 12400–12401, 2004.

Schlesinger, W. H. and Bernhardt, E. S. S.: Biogeochemistry: An Analysis of Global Change, 3rd ed., Academic Press, Waltham, MA., 2013.

Schlickeisen, E., Tietjen, T. E., Arsuffi, T. L. and Groeger, A. W.: Detritus processing and microbial dynamics of an aquatic macrophyte and terrestrial leaf in a thermally constant, spring-fed stream, Microb. Ecol., 45(4), 411–418, 2003.

Sinke, A. J. C., Cornelese, A. A., Cappenberg, T. E. and Zehnder, A. J. B.: Seasonal variation in sulfate reduction and methanogenesis in peaty sediments of eutrophic Lake Loosdrecht, The Netherlands, Biogeochemistry, 16(1), 43–61, 1992.

Steinman, A. D., Lamberti, G. A. and Leavitt, Peter R.: Biomass and pigments of benthic algae, in Methods in Stream Ecology, edited by F. R. Hauer and G. A. Lamberti, pp. 357–380, Academic Press., 2011.

Stumm, W. and Morgan, J. J.: Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters, 3rd ed., John Wiley & Sons, Inc., New York, NY., 1996.

Thauer, R. K.: Biochemistry of methanogenesis: a tribute to Marjory Stephenson, Microbiology, 144, 2377–2406, 1998.

Thilenius, J. F.: Phytosociology and succession on earthquake-uplifted coastal wetlands, Copper River Delta, Alaska, U.S.D.A., Forest Service, Pacific Northwest Research Station., 1995.

Thomas, S. A., Royer, T. V., Snyder, E. B., and Davis, J. C.: Organic carbon spiraling in an Idaho river, Aquat. Sci., 67(4), 424–433, 2005.

Tiegs, S. D., Entrekin, S. A., Reeves, G. H., Kuntzsch, D. and Merritt, R. W.: Litter decomposition, and associated invertebrate communities, in wetland ponds of the Copper River Delta, Alaska (USA), Wetlands, 33(6), 1151–1163, 2013.

U.S. Geological Survey: Largest Rivers in the United States. [online] Available from: http://pubs.usgs.gov/of/1987/ofr87-242/pdf/ofr87242.pdf, 1990.

U.S. Global Climate Change Program: Global climate change impacts in the United States, Cambridge University Press., 2009.

Valentine, D. W., Holland, E. A. and Schimel, D. S.: Ecosystem and physiological controls over methane production in northern wetlands, J. Geophys. Res. Atmospheres, 99(D1), 1563–1571, 1994.

van Dijk, G., Smolders, A. J. P., Loeb, R., Bout, A., Roelofs, J. G. M. and Lamers L. P. M.: Salinization of coastal freshwater wetlands; effects of constant versus fluctuating salinity on sediment biogeochemistry, Biogeochemistry, 126, 71–84, 2015.

Vann, C. D. and Megonigal, J. P.: Elevated CO_2 and water depth regulation of methane emissions: comparison of woody and non-woody wetland plant species, Biogeochemistry 63, 117–134, 2003.

Vermeer, M. and Rahmstorf, S.: Global sea level linked to global temperature, Proc. Natl. Acad. Sci., 106(51), 21527–21532, 2009.

Wagner, M., Roger, A. J., Flax, J. L., Brusseau, G. A. and Stahl, D. A.: Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration, J. Bacteriol., 180(11), 2975–2982, 1998.

Walter, B. P., Heimann, M. and Matthews, E.: Modeling modern methane emissions from natural wetlands: 1. Model description and results, J. Geophys. Res. Atmospheres, 106(D24), 34189–34206, 2001.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J.-M., Hoegh-Guldberg, O. and Bairlein, F.: Ecological responses to recent climate change, Nature, 416(6879), 389–395, 2002.

Weckesser, S., Engel, K., Simon-Haarhaus, B., Wittmer, A., Pelz, K. and Schempp, C. M.: Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance, Phytomedicine, 14(7–8), 508–516, 2007.

West, W. E., Coloso, J. J. and Jones, S. E.: Effects of algal and terrestrial carbon on methane production rates and methanogen community structure in a temperate lake sediment, Freshw. Biol., 57(5), 949–955, 2012.

West, W. E., McCarthy, S. M. and Jones, S. E.: Phytoplankton lipid content influences freshwater lake methanogenesis, Freshw. Biol., 60(11), 2261–2269, 2015.

Westermann, P. and Ahring, B. K.: Dynamics of methane production, sulfate reduction, and denitrification in a permanently waterlogged alder swamp, Appl. Environ. Microbiol., 53(10), 2554–2559, 1987.

Weston, N. B., Vile, M. A., Neubauer, S. C. and Velinsky, D. J.: Accelerated microbial organic matter mineralization following salt-water intrusion into freshwater marsh soils, Biogeochemistry 102(1), 135–151, 2011.

Whalen, S. C.: Biogeochemistry of methane exchange between natural wetlands and the atmosphere, Environ. Eng. Sci., 22(1), 73–94, 2005.

Whiting, G. J. and Chanton, J. P.: Primary production control of methane emission from wetlands, Nature, 364(6440), 794–795, 1993.

Williams, R. T. and Crawford, R. L.: Methane production in Minnesota peatlands, Appl. Environ. Microbiol., 47(6), 1266–1271, 1984.

Winfrey, M. R. and Ward, D. M.: Substrates for sulfate reduction and methane production in intertidal sediments, Appl. Environ. Microbiol., 45(1), 193–199, 1983.

Yannarell, A. C. and Triplett, E. W.: Geographic and environmental sources of variation in lake bacterial community composition, Appl. Environ. Microbiol., 71(1), 227–239, 2005.

Yavitt, J. B. and Seidman-Zager, M.: Methanogenic conditions in northern peat soils, Geomicrobiol. J., 23(2), 119–127, 2006.

Yavitt, J. B., Downey, D. M., Lancaster, E. and Lang, G. E.: Methane consumption in decomposing Sphagnum-derived peat, Soil Biol. Biochem., 22(4), 441–447, 1990.

Yavitt, J. B., Williams, C. J. and Wieder, R. K.: Controls on microbial production of methane and carbon dioxide in three Sphagnum-dominated peatland ecosystems as revealed by a reciprocal field peat transplant experiment, Geomicrobiol. J., 17(1), 61–88, 2000.

Zar, J. H.: Biostatistical Analysis, 5th ed., Pearson Prentice-Hall, Upper Saddle River., 2010.

Zverlov, V., Klein, M., Lücker, S., Friedrich, M. W., Kellermann, J., Stahl, D. A., Loy, A. and Wagner, M.: Lateral gene transfer of dissimilatory (bi)sulfite reductase revisited, J. Bacteriol., 187(6), 2203–2208, 2005.

Table 1. Water column physical and chemical characteristics (mean ± sd) of the wetlands sampled in the Copper River Delta including elevation, depth, temperature, pH, specific conductivity (SpC), salinity, dissolved organic carbon (DOC), and sulfate concentrations. For the freshwater wetlands, physicochemical parameters are from spot measurements of the hypolimnion conducted throughout summer 2014 (n = 4 per freshwater wetland), whereas DOC and sulfate were measured in the surface layer in June and August 2014 (5 sites x 2 time periods, n =10 per wetland). For the brackish wetlands, physicochemical parameters are from one spot measurement of the surface layer. Tidal brackish wetlands A–E were sampled in June and F–J were sampled in August.

Wetland	Elevation (m)	Depth (m)	Temp (°C)	pH	SpC (µs cm^{-1})	Salinity (ppt)	DOC (mg L^{-1})	Sulfate (µM)
Eyak N	5.2	0.60 ± 0.09	15.3 ± 0.9	5.5 ± 0.4	13 ± 3	0.01 ± 0.01	6.5 ± 1.9	1.6 ± 0.3
Eyak S	5.5	0.61 ± 0.03	16.1 ± 1.3	7.0 ± 1.1	11 ± 2	0.00 ± 0.01	5.6 ± 0.5	2.0 ± 0.2
Lily	8.2	0.65 ± 0.04	13.1 ± 0.8	5.9 ± 0.2	60 ± 19	0.03 ± 0.01	3.5 ± 1.0	6.0 ± 2.2
Rich Hate Me	18.3	0.57 ± 0.15	11.6 ± 2.9	6.1 ± 0.4	56 ± 7	0.03 ± 0.01	2.1 ± 0.5	24 ± 5
Scott S	13.4	0.81 ± 0.07	14.2 ± 0.9	6.3 ± 0.3	61 ± 37	0.03 ± 0.02	2.1 ± 0.6	54 ± 17
Storey N	4.6	0.56 ± 0.04	16.8 ± 1.0	6.9 ± 0.4	74 ± 11	0.04 ± 0.01	11 ± 0.6	4.5 ± 0.7
Storey S	2.1	0.60 ± 0.04	16.6 ± 2.4	7.3 ± 0.7	70 ± 6	0.03 ± 0.00	4.2 ± 0.3	7.9 ± 0.5
Tiedeman N	5.5	0.66 ± 0.03	16.6 ± 1.1	6.0 ± 0.5	13 ± 3	0.01 ± 0.01	6.7 ± 0.7	1.8 ± 0.2
Tiedeman S	5.5	0.73 ± 0.03	15.4 ± 1.4	6.7 ± 0.6	8.8 ± 1.7	0.00 ± 0.00	5.2 ± 0.6	1.7 ± 0.3
Brackish A	2.4	0.22	14.9	6.9	190	0.09	0.79	130
Brackish B	2.7	0.30	17.6	7.6	19000	11	0.97	8100
Brackish C	0	0.38	17.4	7.8	20000	12	1.2	8900
Brackish D	0	0.49	16.0	7.6	570	0.28	0.68	37
Brackish E	0	0.35	15.3	7.6	2100	1.1	0.67	1100
Brackish F	0.6	0.73	11.3	6.4	1500	0.78	0.88	250
Brackish G	2.4	0.78	12.2	6.8	160	0.07	0.78	11
Brackish H	2.7	0.65	12.9	7.5	13000	7.7	1.7	6000
Brackish I	0.3	0.80	12.2	7.5	24000	15	2.2	8300
Brackish J	2.7	0.89	13.1	7.6	3800	2.0	21	930

Table 2. Sediment chemical characteristics (mean ± sd) of the wetlands in the Copper River Delta including sediment organic matter (SOM %), total sediment carbon, and porewater (PW) concentrations of acetate, nitrate, and sulfate as well as total sulfate availability in the slurry incubations. For the freshwater wetlands, measurements were conducted in June and August 2014 (5 sites x 2 time periods, n =10 per wetland). For the brackish wetlands, sediment chemistry is from one measurement only. Tidal brackish wetlands A–E were sampled in June and F–J were sampled in August. All chemistry parameters were converted to the total amount of anion per gram of dry sediment (nmol g^{-1}) for analyses, but standard porewater concentrations (µM) are also reported for comparison with other studies. Porewater nitrate concentrations were extremely low with many below what we considered our detection limit (i.e., < 2 µM).

Wetland	SOM (%)	Total Sediment C (mmol g^{-1})	PW Acetate (nmol g^{-1}) / (µM)		PW Nitrate (nmol g^{-1}) / (µM)		PW Sulfate (nmol g^{-1}) / (µM)		Total Sulfate (nmol g^{-1})
Eyak N	2.0 ± 0.5	0.81 ± 0.22	57 ± 57	360 ± 350	1.2 ± 0.7	9.6 ± 9.9	150 ± 160	970 ± 950	160 ± 160
Eyak S	1.8 ± 0.5	0.71 ± 0.21	18 ± 13	120 ± 82	1.4 ± 1.0	4.3 ± 3.1	65 ± 48	500 ± 350	67 ± 48
Lily	2.1 ± 0.5	0.86 ± 0.21	58 ± 43	620 ± 560	0.86 ± 0.26	0.4 ± 0.2	5.1 ± 3.2	49 ± 27	11 ± 3
Rich Hate Me	3.1 ± 3.9	1.3 ± 1.6	29 ± 44	110 ± 140	3.7 ± 4.9	2.2 ± 4.2	60 ± 130	96 ± 110	84 ± 140
Scott S	1.5 ± 2.2	0.60 ± 0.89	31 ± 34	300 ± 340	2.2 ± 2.4	0.8 ± 0.8	11 ± 11	120 ± 110	51 ± 14
Storey N	1.8 ± 0.2	0.73 ± 0.09	10 ± 5	120 ± 64	0.92 ± 0.39	1.2 ± 0.8	18 ± 11	210 ± 160	22 ± 12
Storey S	1.9 ± 3.1	0.76 ± 1.2	15 ± 16	160 ± 120	0.58 ± 0.39	2.8 ± 3.1	39 ± 44	450 ± 460	46 ± 45
Tiedeman N	2.8 ± 2.9	1.1 ± 1.2	25 ± 14	190 ± 95	1.7 ± 1.2	3.7 ± 2.8	56 ± 43	420 ± 320	93 ± 81
Tiedeman S	2.3 ± 0.8	0.93 ± 0.32	17 ± 7	120 ± 45	2.1 ± 1.9	4.3 ± 3.4	66 ± 53	440 ± 320	67 ± 53
Brackish A	14	5.5	240	2500	1.2	12	660	6900	770
Brackish B	1.7	0.70	72	230	0.03	0.10	3000	9500	8900
Brackish C	10	4.1	180	2400	0.01	0.10	1200	15000	9000
Brackish D	2.3	0.94	61	950	0.01	0.10	630	9900	660
Brackish E	1.9	0.75	110	1000	0.01	0.10	1100	10000	2100
Brackish F	7.3	2.9	140	1500	0.01	0.10	540	5900	740
Brackish G	3.3	1.3	660	6400	0.01	0.10	430	4200	440
Brackish H	9.0	3.6	200	1500	0.01	0.10	2500	19000	7600
Brackish I	13	5.3	38	550	0.01	0.10	1200	17000	7500
Brackish J	1.3	0.54	25	380	0.01	0.10	670	10000	1400

Table 3. Generalized linear models (GLMs) wherein log-transformed CH_4 production rate is the response variable and ecosystem type (non-tidal freshwater or tidal brackish), time period (June or August), porewater acetate level, and total sulfate availability are potential factors. Positive (↑) or negative effects (↓) of continuous factors are indicated. Models are ranked in order of the lowest Akaike information criterion corrected for low samples sizes (AIC_c) along with delta AIC_c (Δ_i) and Akaike weights (ω_i) before and after model averaging (MA). Models with a Δ_i larger than 4 were not included in the model averaging. The three models with a larger AIC_c than the null model (intercept only) are not presented.

Model #	GLM	AIC_c	Δ_i	ω_i	$\omega_{i\ (MA)}$
1	ecosystem + time period + acetate (↑) + sulfate (↓)	125.3	0.0	0.571	0.61
2	ecosystem + acetate (↑) + sulfate (↓)	127.0	1.7	0.244	0.26
3	ecosystem + time period + acetate (↑)	128.4	3.1	0.120	0.13
4	ecosystem + acetate (↑)	129.7	4.4	0.062	-
5	ecosystem + time period + sulfate (↓)	137.7	12.4	0.001	-
6	ecosystem + sulfate (↓)	139.2	13.9	0.001	-
7	time period + sulfate (↓)	140.2	14.9	0	-
8	sulfate (↓)	140.9	15.6	0	-
9	time period + acetate (↑) + sulfate (↓)	141.4	16.2	0	-
10	acetate (↑) + sulfate (↓)	141.4	16.2	0	-
11	ecosystem + time period	142.9	17.6	0	-
12	ecosystem	144.2	19.0	0	-
13	null	158.2	33.9	0	-

Table 4. Generalized linear models (GLMs) wherein ΔCH_4 production rate (treatment minus control) is the response variable and the macrophyte species added (buckbean, horsetail, lily, or marestail), porewater acetate availability, and total sulfate availability are potential factors. Positive (↑) or negative effects (↓) of continuous factors are indicated. Models are ranked in order of the lowest Akaike information criterion corrected for low samples sizes (AIC_c) along with delta AIC_c (Δ_i) and Akaike weights (ω_i) before and after model averaging (MA). The null model (intercept only) was not included in the model averaging, and the three models with a larger AIC_c than the null model are not presented.

Model #	**GLM**	**AIC_c**	**Δ_i**	**ω_i**	**$\omega_{i\ (MA)}$**
1	acetate (↓)	286.4	0.0	0.429	0.52
2	acetate (↓) + species	288.2	1.8	0.178	0.22
3	acetate (↓) + sulfate (↑)	288.9	2.5	0.121	0.15
4	species	289.4	3.0	0.096	0.12
5	null	290.0	3.6	0.072	-

Figure Captions

Figure 1. Conceptual diagram illustrating the potential effects of warming, sea-level rise, and increased organic matter (OM) availability on CH_4 production in coastal wetlands. These three global change mechanisms are all indirect consequences of rising CO_2 levels.

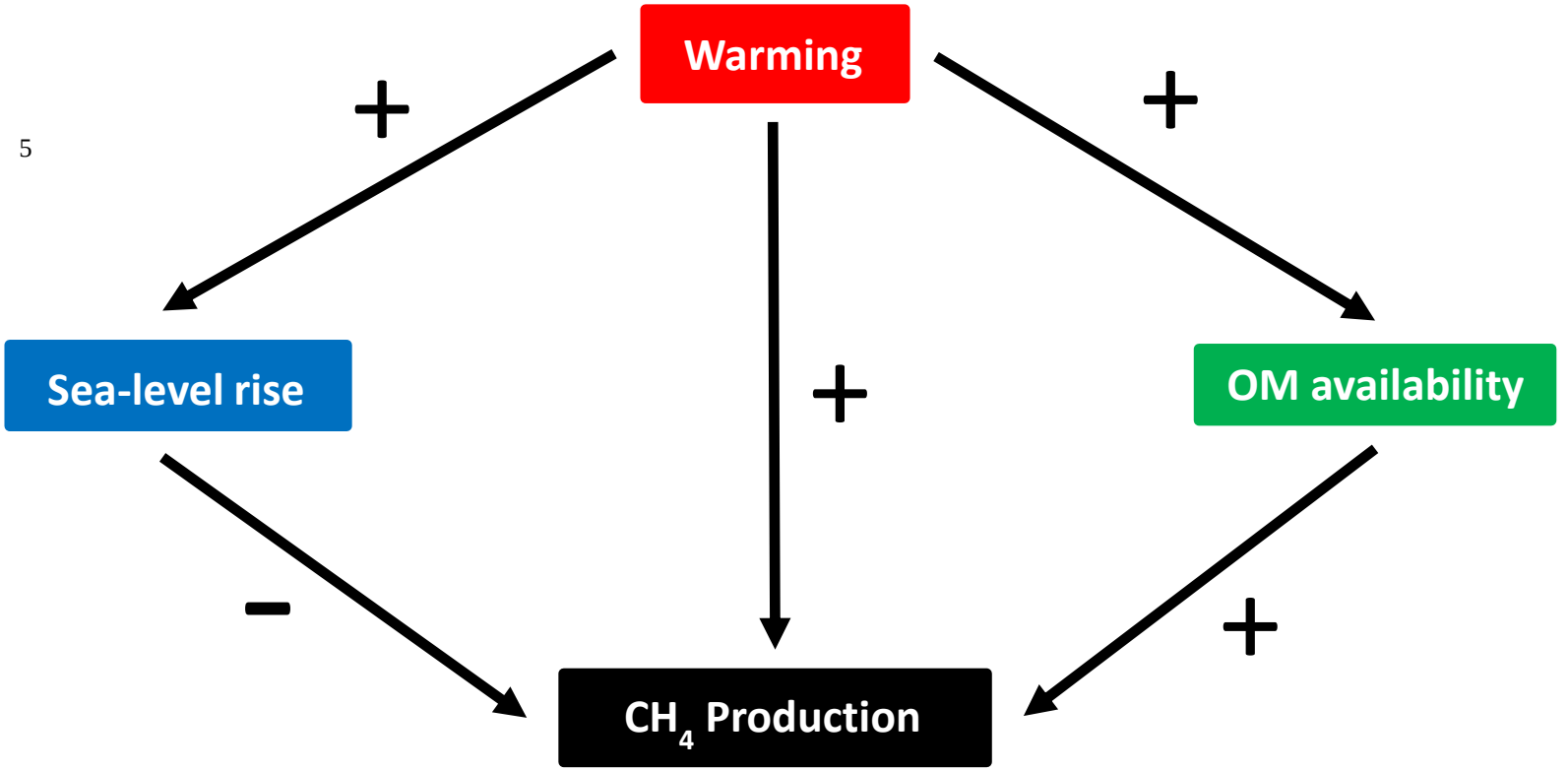
Figure 2. Aerial photo of the Copper River Delta taken by the USDA Forest Service depicting the major wetland ecosystem types extending from glaciers to ocean.

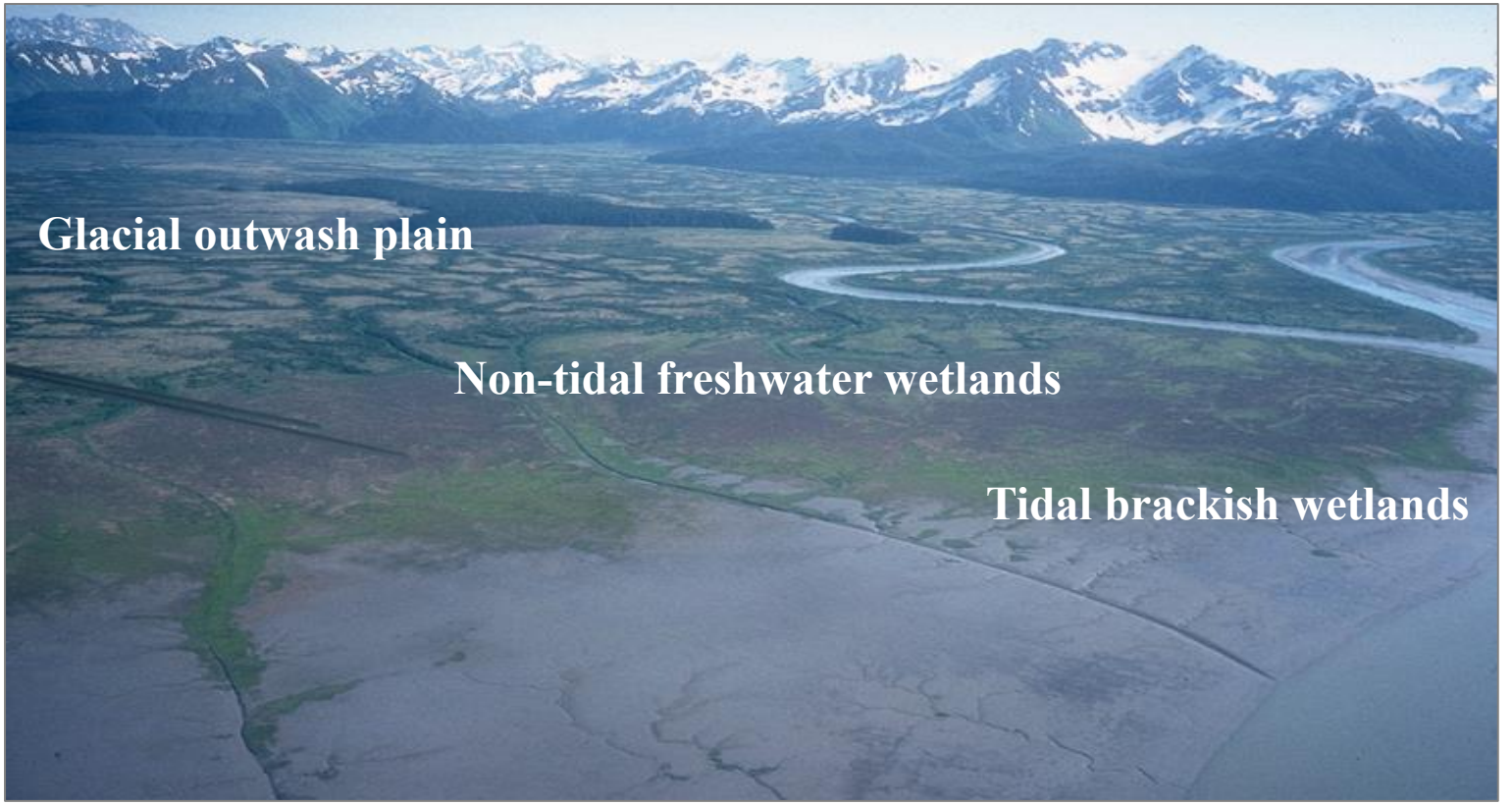
Figure 3. Mean CH_4 production rates (nmol g^{-1} of dry sediment day^{-1}) from Copper River Delta non-tidal freshwater (n = 9) and tidal brackish (n = 5) wetlands during A) June and B) August, 2014. Error bars represent standard errors.

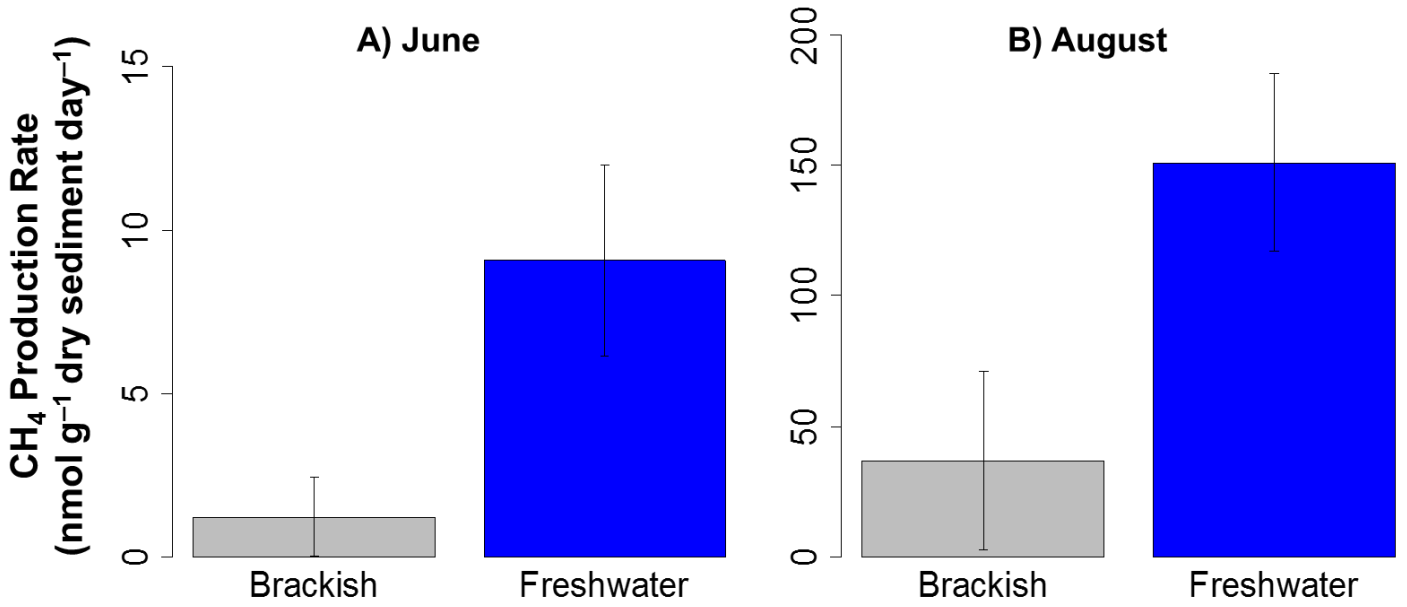
Figure 4. Mean CH_4 production rates (nmol g^{-1} of dry sediment day^{-1}) from non-tidal freshwater wetland sediments incubated with freshwater (FW/FW; n = 5) and other sediments from the same freshwater wetlands incubated with brackish water from tidal brackish wetlands (FW/BR; n = 5). Error bars represent standard errors. This seawater addition experiment was conducted over a 14-day period in June 2014.

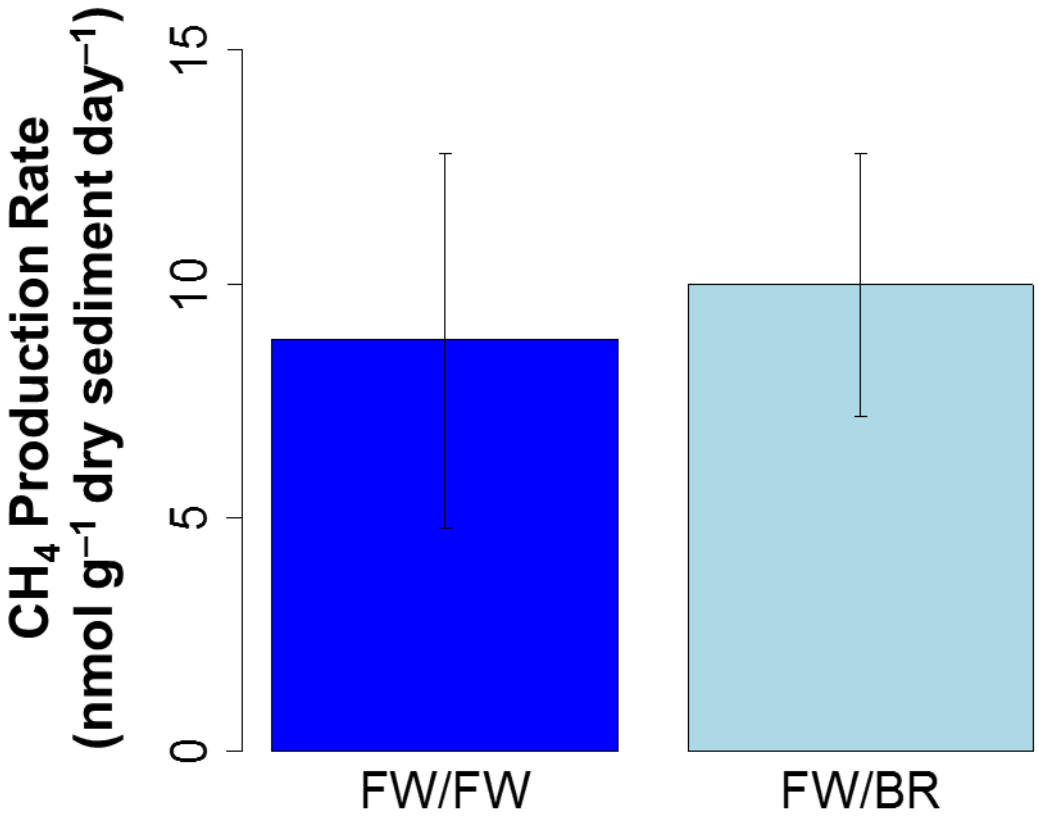
Figure 5. Mean CH_4 production rates (nmol g^{-1} of dry sediment day^{-1}) from organic matter treatments (CTL = control, BB = buckbean *Menyanthes trifoliata*, HT = horsetail *Equisetum variegatum*, LI = lily *Nuphar polysepalum*, and MT = marestail *Hippuris vulgaris*) replicated in five non-tidal freshwater wetlands during August 2014. Error bars represent standard error.

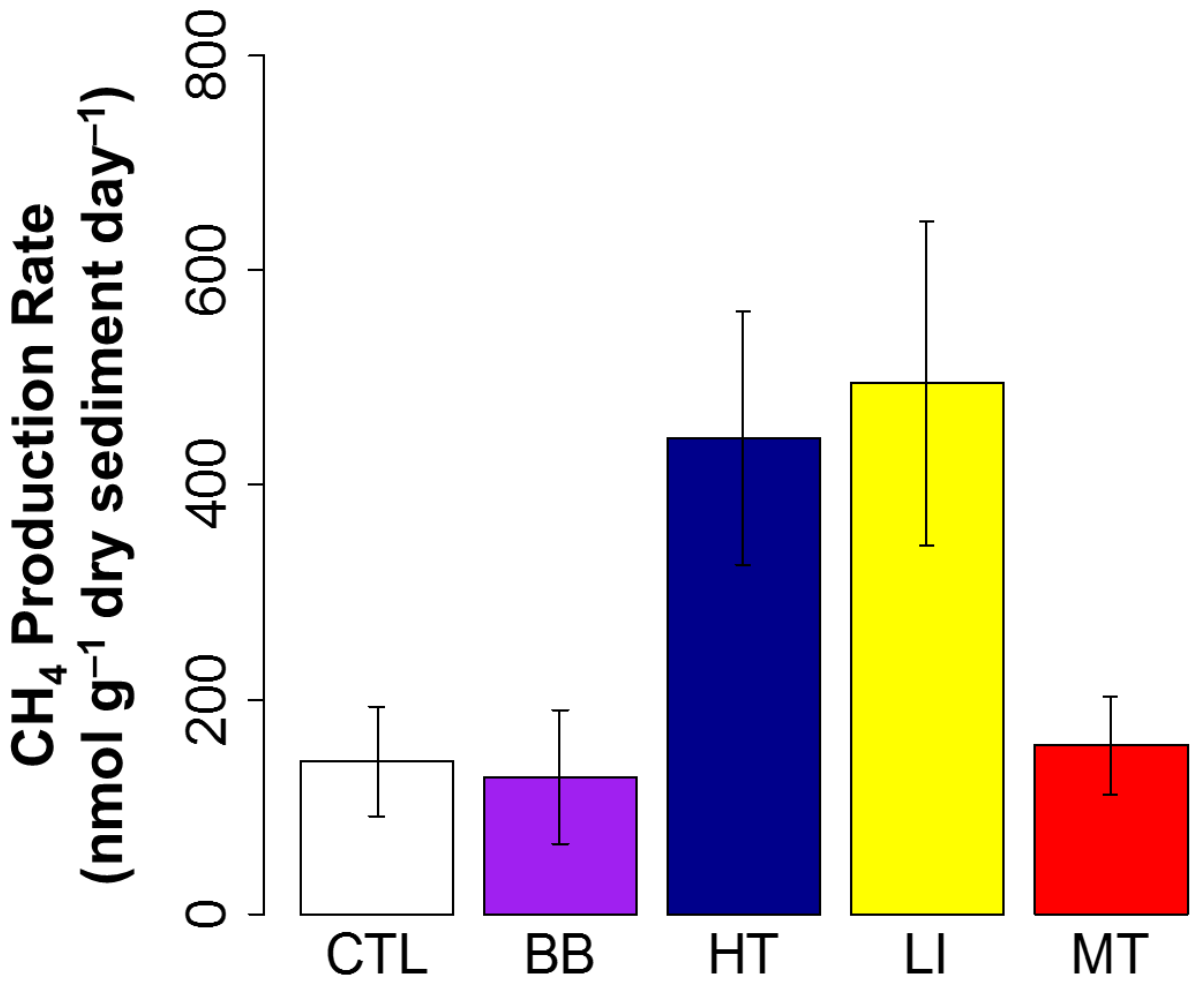
Figure 6. Actual response of ΔCH_4 production (treatment–control; nmol g^{-1} of dry sediment day^{-1}) plotted against the predicted response from model-averaged parameter estimates of the macrophyte species added (BB= buckbean *Menyanthes trifoliata*, HT = horsetail *Equisetum variegatum*, LI = lily *Nuphar polysepalum*, and MT = marestail *Hippuris vulgaris*), porewater acetate availability, and total sulfate availability. The dashed black line depicts the 1:1 line, and above the gray dotted line marks the point at which adding organic matter increased CH_4 production (or ΔCH_4 production > 0). The solid black line is the best-fit line between the actual and the predicted responses ($y = 0.95x - 86$; r^2 of 0.59), which demonstrates that although the model did a decent job of predicting relative changes in the response, it tended to underestimate ΔCH_4 production.

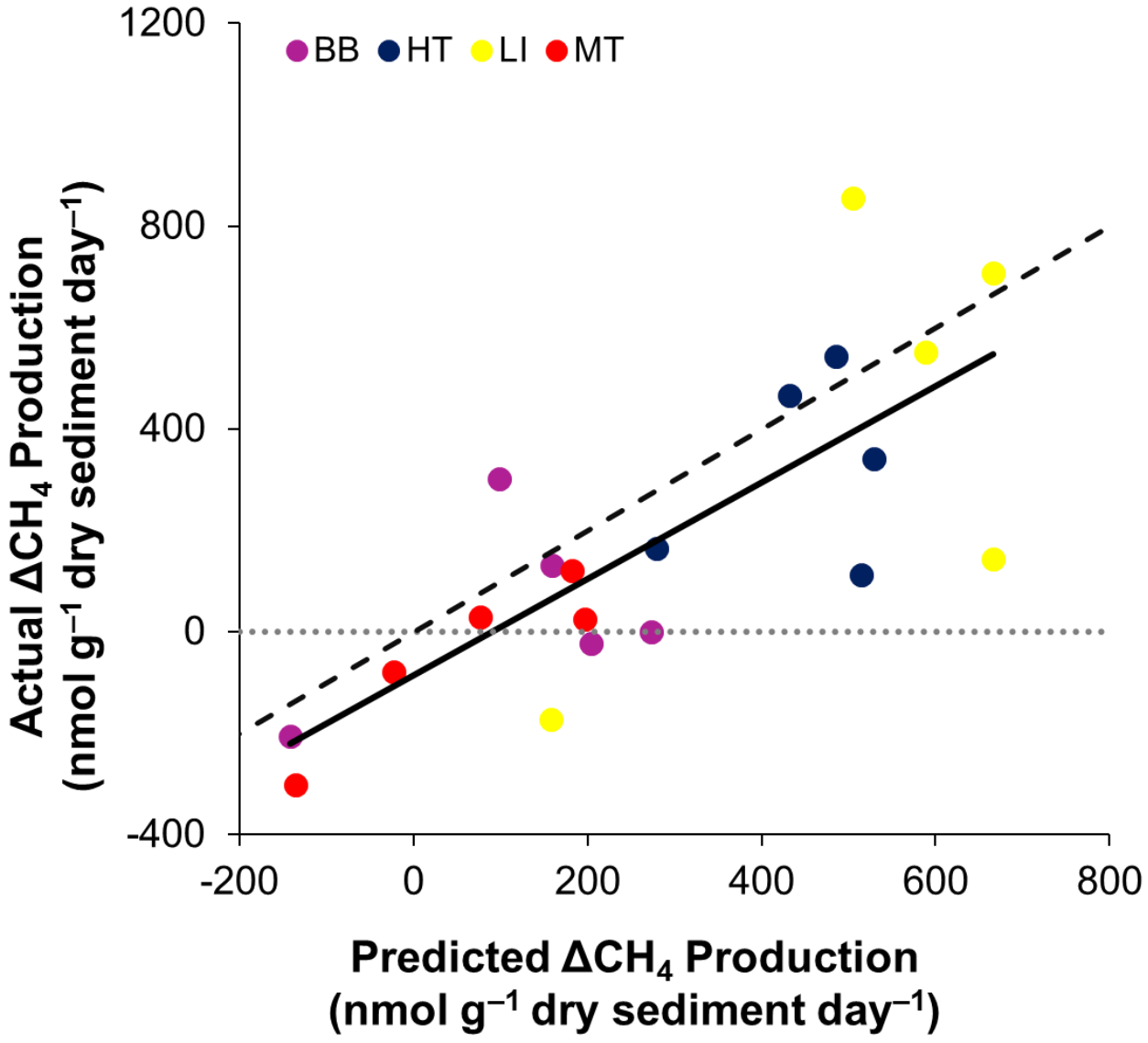

Warming
+
+
Sea-level rise
+
OM availability
−
+
CH4 Production


Glacial outwash plain
Non-tidal freshwater wetlands
Tidal brackish wetlands


A) June
B) August
CH_4 Production Rate
(nmol g^{-1} dry sediment day^{-1})
0
5
10
15
50
100
150
200
Brackish
Freshwater
Brackish
Freshwater


CH_4 Production Rate
(nmol g^{-1} dry sediment day^{-1})
0
5
10
15
FW/FW
FW/BR


CH_4 Production Rate
(nmol g^{-1} dry sediment day^{-1})
0
200
400
600
800
CTL
BB
HT
LI
MT


BB
HT
LI
MT
1200
800
400
0
-400
-200
0
200
400
600
800
Actual ΔCH4 Production
(nmol g−1 dry sediment day−1)
Predicted ΔCH4 Production
(nmol g−1 dry sediment day−1)